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A STUDY OF CERTAIN CHARACTERS IN WHEAT BACK CROSSES [1]

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INTRODUCTION

A wheat-crossing program was undertaken in the spring of 1925 to improve the milling qualities of the club wheats. The club wheats have definite characteristics which make them especially well adapted to certain areas. They yield well, are stiff strawed, and resistant to lodging and shattering. Their ability under normal conditions to stand erect for a considerable time after maturity without appreciable loss of grain makes them well suited to grain-growing areas where the combined harvester is used for harvesting. Because of these characteristics the club wheats have become important in the Pacific Northwest, particularly in certain sections of Oregon, Washington, and adjacent parts of Idaho. They are grown to a considerable extent also in certain localities in California, where serious losses often occur in wheats less resistant to shattering. However, the soft kernels and the comparatively low milling value of the club wheats, usually reflected in lower market prices, make them somewhat unsatisfactory to the grower.

In an attempt to improve the milling value (quality), two high-yielding club varieties were crossed in various combinations with three varieties of common wheat having good bread-making qualities. High milling and baking quality in wheat is a complex character. In order to obtain this quality more readily in the club segregates, back crossing was resorted to.

With the aid of a recently developed technic for producing wheat crosses, a genetic study on this material was undertaken in order to obtain further information as to the use of the back cross in genetic analyses of characters in wheat, with special reference to the inheritance of the club type of spike. The data on back crossing were checked in part by a collateral study of the usual F_2 and F_3 generations.

The following pairs of contrasting characters were studied: Club spike and lax spike, in four crosses; brown chaff and white chaff, in one cross; and dwarf plants and normal plants and early maturity and late maturity, in two crosses each.

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BACK CROSSING IN WHEAT BREEDING

The value of the back cross in economic cereal-breeding problems was called to the attention of plant breeders in 1922 by Harlan and Pope (*8*)[3] in their work with barley. More recently the importance of this method of intensifying desirable characters has become more widely recognized by plant breeders. Briggs (*2*) has reported on the use of this method in an attempt to transfer bunt resistance to commercial varieties of wheat. By repeated back crossing it is possible almost to duplicate a given variety, at the same time retaining the factor carrying the desired new character brought in by the cross.

The back cross is a valuable aid in factor analysis, but it should not be thought of as supplanting the usual method applied to the regular filial generations. Character relations, such as are found in the case of cumulative color factors in wheat, may be determined at once by the type of segregations obtained in the F_3, which ordinarily could not be shown by the back-cross method.

The back-cross method might be useful in the study of quantitative characters, such as earliness, height, and rust and smut reaction, which are not satisfactorily analyzed by the usual method.

MATERIAL AND METHODS

DESCRIPTION OF VARIETIES

Parent plants were selected from pure-line varieties that had been under observation by the writer for a number of years before the crosses were made. The varieties used in these tests were Little Club, Jenkin, Quality, Hard Federation, and Marquis.

Little Club (*Triticum compactum* Host., C. I.[4] 4066) (*4*) is a late-maturing spring-habit wheat with white soft kernels and white chaff. It has moderately short strong straw and medium short awnless spikes whose average rachis-internode length is about 2.1 to 2.2 mm.

Jenkin (*Triticum compactum* Host., C. I. 5177) (*4*), an important commercial variety in the Pacific Northwest and one of the highest yielding varieties at Davis, Calif., is a late-maturing spring-habit wheat with white, medium large, soft kernels and brown chaff. It has tall, strong, white straw and awnless spikes. The average rachis-internode length is about 2.7 to 2.8 mm.

Quality (*Triticum vulgare* Vill., C. I. 6157) (*4*) is a low yielder in California and shatters badly, but it produces grain of excellent milling and baking quality. It is an early-maturing, spring-habit variety with hard white kernels. It has short, moderately strong, white straw and white chaff. Except for a few apical awns the spike is awnless. The average length of rachis internodes varies from about 4.2 to 4.5 mm.

Hard Federation (*Triticum vulgare* Vill., C. I. 4733) (*4*) has produced consistently high yields in experiments at Davis, and its grain possesses high bread-making qualities. It is an early maturing spring-habit wheat with short, hard, white kernels, brown chaff, and short, strong, white straw. The spike is awnless, rather square in cross section, and medium in density. The average length of rachis internodes is approximately 4.2 to 4.4 mm.

[3] Reference is made by number (italic) to Literature Cited, p. 498.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

Marquis (*Triticum vulgare* Vill., C. I. 4158) (*4*), the well-known hard red spring wheat, is grown commercially only to a limited extent in California. Its excellent bread-making qualities are well known. Marquis is a spring-habit wheat with short, hard, red kernels, white chaff, medium-tall white straw, and apically awnleted spikes. The average length of rachis internodes is approximately 4.3 to 4.5 mm. Marquis is midseason to late in maturity in California.

EXPERIMENTAL METHODS

The following crosses and their reciprocals were made in May, 1925: Quality × Little Club; Quality × Jenkin; Jenkin × Hard Federation; and Jenkin × Marquis. The F_1 hybrids were back-crossed to both parents except in the last cross, which was back-crossed only with Marquis. The F_1 plants used in back crossing provided material for study in the usual F_1, F_2, and F_3 generations, except that in Quality × Jenkin the reciprocal cross was used.

The wheat back crosses were obtained by crossing the F_1 of each cross with both parents. The back crosses were made to both parents,

FIGURE 1.—Bagged back-crossed spikes of F_1 plants of common wheat × club wheat crosses at maturity. The shelters were erected to protect the plants from wind injury

as each parent carried recessive characters which were to be studied. It was also desired to intensify both the *compactum* (club) and the hard-texture or high-protein factors in order to facilitate subsequent selection.

Vigorous F_1 plants were secured as material for back crossing in the spring of 1926. Supplies of pollen were procured from rows of pure-line parental material which had been sown at monthly intervals on three different dates. The usual precautions were observed in all hybridizing operations. Back-crossed heads are shown in Figure 1.

Seeds from the first-year crosses and their parents were sown 3 to 4 inches apart in 3 to 5 foot rows 1 foot apart. Second-year back-crossed kernels, F_1, F_2, and F_3 material, and seeds of the parents were sown 1½ to 2½ inches apart in 16-foot rows 1 foot apart. Ends and unoccupied portions of rows were sown to an easily distinguishable variety of wheat to reduce border effect.

Density of spike was studied by rachis-internode measurements and also by inspection. As will be seen hereafter, length of rachis internode alone does not always correctly separate the two classes club and lax (common), so that some overlapping results. Only the leading spike of the plant was used for measurements.

The rachis-internode length was determined by measuring in millimeters the length of 10 internodes, the third to the twelfth, inclusive, from the base of the spike. The two lower internodes were not included in the measurements because their lengths were too inconstant. Club spikes are more dense toward the tip, and lax spikes are more dense toward the base. Similar internode relations in barley were noted by Hayes and Harlan (*10*). By eliminating the lower internodes it is believed that comparable measurements were obtained.

Earliness was determined by noting the date of first exsertion of the spike from the top of the sheath. To properly differentiate plants coming into head a rubber band was slipped over each plant. On the date of first heading the plant was tagged and the rubber band removed.

Color of chaff was determined by inspection, specimens of the parent plants being used as a standard of comparison.

Those plants were considered dwarfs which were much reduced in stature and which because of shortened culm internodes were leafy or grasslike at the base. In some instances such plants failed to shoot at all and remained entirely grassy, for which reason they are termed "grassy" plants.

To expedite the experiment, kernels of the original crosses were sown in July, 1925, as a late summer crop at Palo Alto, Calif. Seed of excellent quality was produced by all hybrids and parents with the exception of Jenkin and Little Club. This F_1 seed was sown at Davis, Calif., in December of the same year.

EXPERIMENTAL DATA

The experimental data fall into two groups: (1) That covering the back-crossed generations, and (2) that covering the F_1, F_2, and F_3 generations of the original crosses. The type of spike was studied in both kinds of material in all crosses, color of glumes in one cross, and dwarfing in two crosses. Earliness was studied only in the back-crossed generation of two crosses.

THE F_1 GENERATION

Characters of the F_1 plants used in back crossing and of their parents are presented in Table 1. The F_1 spikes of all crosses were classified as long club. The heads were longest in Jenkin × Hard Federation and Jenkin × Marquis. The average length of rachis internodes was strongly inclined toward the club parent in three of the crosses, but in Jenkin × Hard Federation it was intermediate between the parents.

In two crosses involving brown-chaffed and white-chaffed parents, the F_1 plants were brown chaffed but of a lighter shade than the brown parent. The F_1 kernels of Jenkin × Marquis were red but of a somewhat lighter shade than that of the Marquis parent.

Dates of first heading were not recorded for the F_1 hybrid plants, but dates of first ripening indicated that all crosses were intermediate to their parents in maturity. No dwarfing was observed in the F_1 generation.

TABLE 1.—*Characters of F_1 plants of four common×club wheat crosses used in a wheat back-cross experiment, 1926*

Cross or parent	F_1 plants measured	Average rachis-internode length	Spike type	Glume color	Kernel color	Date	
						First headed	First ripe
	Number	*Mm.*					
Quality×Little Club	7	2.9	Long club	White	White		May 27
Do	8	2.8	do	do	do		Do.
Quality×Jenkin	14	3.1	do	Light brown	do		May 29
Jenkin×Hard Federation	11	3.7	do	Brown	do		
Hard Federation×Jenkin	15	3.7	do	do	do		May 28
Jenkin×Marquis	9	3.4	do	Light brown	Red		
Quality	6	5.0	Lax	White	White	Apr. 2	May 21
Little Club	3	2.4	Short to mid-long club.	do	do	Apr. 20	June 3
Jenkin	9	3.0	Long club	Brown	do	do	June 5
Marquis	7	4.4	Long lax	White	Red	Apr. 25	June 1
Hard Federation	6	4.6	Square, short lax.	Brown	White	Mar. 31	May 18

A study of the F_1 plants grown at Palo Alto showed them to be of apparently identical genetic constitution with those described in Table 2.

THE WHEAT BACK CROSSES

A list of the back crosses showing the number of heads crossed and kernels obtained is given in Table 2.

A total of 4,692 kernels was obtained from 231 crossed heads. These heads were grown on 52 F_1 plants. Some of the plants were back crossed with both parents. This explains the apparent discrepancy between the total number of plants listed as used in back crossing and the number of plants actually used.

TABLE 2.—*Wheat back crosses made in 1926 and used for character study in the back-crossed generation*

Back cross	F_1 plants used in back crossing	Crossed heads	Kernels obtained
	Number	*Number*	*Number*
(Quality×Little Club)×Little Club	9	28	494
(Quality×Little Club)×Quality	13	45	1,013
(Quality×Jenkin)×Quality	12	40	952
(Quality×Jenkin)×Jenkin	6	42	457
(Jenkin×Hard Federation)×Jenkin	17	28	579
(Jenkin×Hard Federation)×Hard Federation	23	42	1,007
(Jenkin×Marquis)×Marquis	3	6	190
Total		231	4,692

THE POLLEN-PARENT PURE LINES

To check the pollen-parent material used in back crossing for uniformity of spike type and other characters, seed from each of the pure-line parents was sown in five rows a rod long and seed from first-generation plant selections from parent progenies was sown in an equal number of rows.

Mean rachis-internode lengths, standard deviations, and coefficients of variation of parent pure lines used as sources of pollen in back crossing, and pure lines from original parents are shown in Table 3.

The density distributions of the bulked pure lines, as compared with those of the pure lines from the original parent-plant material, were strikingly alike in every case. The range of variation is almost identical, while the modes of the distribution also are identical in most cases. Such variations as do occur may be attributed to the small number of individuals used in certain of the populations.

TABLE 3.—*Mean rachis-internode length, standard deviation, and coefficient of variation of parent pure lines used as sources of pollen in back crossing, and of plant progenies from original parents, 1927*

Parent		Mean rachis-internode length	Standard deviation	Coefficient of variation	Plants
Pure line (bulk seed)	Pure-line plant and selection No.				
		Mm.	*Mm.*		*Number*
Hard Federation		4.39±0.03	0.56	12.8	208
	Hard Federation 34–1	4.31± .03	.49	11.4	136
Quality		4.43± .02	.53	12.0	290
	Quality 22–1	4.51± .02	.64	14.2	311
Jenkin		2.64± .01	.30	11.4	227
	Jenkin 29–5	2.72± .02	.32	11.8	140
Little Club		2.25± .01	.27	12.0	251
	Little Club 26–2	2.29± .02	.25	10.9	58
Marquis		4.09± .03	.46	11.2	161
	Marquis 33–1	4.19± .03	.45	10.7	129
Hard Federation [a]		4.69	.41	8.7	39
Do. [b]		4.43	.38	8.6	65

[a] Normal [b] Infected with foot rot.

From these data it is concluded that the results obtained from the character studies in the back-crossed generation probably are as reliable as if progeny of the actual parent plants had been used for the back-cross pollinations.

Hard Federation wheat is susceptible to foot rot in wet seasons. The bulked population of this variety grown in two rows (Table 3) was segregated into normal and foot-rotted plants to determine the effect of this disease on rachis-internode length. Rachis-internode length was reduced below the lower limit of the normal plants in about 17 per cent of the foot-rotted plants. Diseased plants were eliminated from density measurements wherever possible, but those included probably did not seriously change the data.

SEGREGATION OF CHARACTERS

DENSITY OF SPIKE

Investigators agree that density of spike in wheat is controlled primarily by a single factor and that the club factor is dominant. Nilsson-Ehle (*12*) obtained a ratio of approximately 15 long lax to 1 squarehead among the lax segregates of the cross club (*compactum*) × squarehead. Likewise he observed different densities among the club segregates, including those more dense than the club parent. He assumed a dominant length-inhibiting factor C to be responsible for the club (*compactum*) type of spike, and the dominant (in the absence of C) internode-lengthening factors L_1, L_2. The genotype of the club parent used in his cross was $CCL_1L_1L_2L_2$ and that of the squarehead parent $ccl_1l_1l_2l_2$.

Parker (*13*) found a 1-factor difference in a *compactum* × *vulgare* cross. He states that his results did not confirm Nilsson-Ehle's hypothesis as to multiple factors for density.

Meyer (*11*) found a bifactorial difference in *vulgare* hybrids involving crosses between the comparatively dense squarehead type of wheats and the lax, long-spiked varieties.

Stewart (*15*) found a ratio of 1 dense to 2 heterozygous to 1 lax in his studies of spike density in wheat hybrids. He concluded that no genetic hypothesis has been found—

> that will explain the fact of wide transgressive segregation of spike density in both directions and still account for the very infrequent recovery of the spike density of one of the parents.

Nilsson-Ehle (*12*) found in crosses between club-spiked and lax-spiked wheats that the club segregates had a density of 1.4 to 2.2 mm.

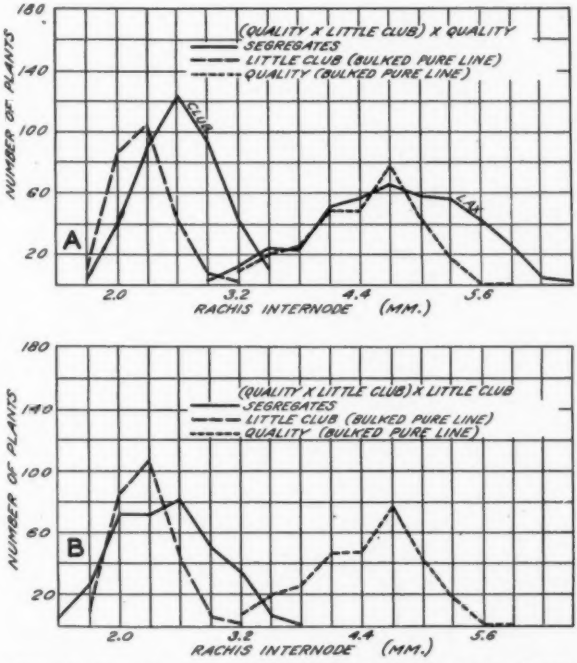

FIGURE 2.—Curves for spike density in wheat back crosses: A, (Quality × Little Club) × Quality; B, (Quality × Little Club) × Little Club

Boshnakian (*1*) states that the internode length of dense wheats varies from 1.2 to 2.5 mm. and that of common wheats from 3 to 5 mm.

SPIKE DENSITY IN BACK CROSSES

Frequency distribution of average rachis-internode lengths of parents and back crosses is given in Table 4. Curves showing the distribution of internode lengths in populations of back crosses with both parents for three crosses and in a back cross with Marquis alone are shown in Figures 2 to 5, inclusive. Internode classes are plotted on the abscissas and the number of individuals on the ordinates. For back crosses with the lax parent, spike-density figures are given

TABLE 4.—*Frequency distribution of average rachis-internode lengths by 0.3 mm. classes of wheat back-cross populations and parent varieties, 1927*

Parent, selection No., back cross cross, and type of spike	Number having average rachis-internode length (0.3 mm. classes) indicated																					Plants	Rachis-internode length		
	1.4	1.7	2.0	2.3	2.6	2.9	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9	6.2	6.5	6.8	7.1	7.4	7.7		Mean	Standard deviation	Coefficient of variation
																							Number	*Mm.*	*Mm.*	
Hard Federation (bulked pure line)						5	5	12	24	28	44	44	35	9	2								208	4.39	0.56	12.8
Hard Federation 34–1						1	4	10	13	32	25	37	11	2	1								136	4.31	.49	11.4
Quality (bulked pure line)							8	20	26	48	48	78	43	17	1	1							290	4.43	.53	12.0
Quality 22–1						1	9	29	28	39	46	52	54	34	18	1							311	4.51	.64	14.2
Jenkin (bulked pure line)			10	46	90	65	14	2															227	2.64	.30	11.4
Jenkin 29–5		1	4	20	48	51	13	1	2														140	2.72	.32	11.8
Little Club (bulked pure line)		10	85	105	42	7	2																251	2.25	.27	12.0
Little Club 26–2		1	15	28	12	2																	58	2.29	.25	10.9
Marquis (bulked pure line)						3	7	15	36	43	36	11	7	2	1								161	4.09	.46	11.2
Marquis 33–1							7	10	20	29	34	23	6										129	4.19	.45	10.7
(Quality×Little Club)×Little Club	Club	5	27	72	72	82	51	35	6	1														351	2.45	.47	19.2
	Lax								3	1	2	1	2		1	3							13			
(Quality×Little Club)×Quality [a]	Club		5	41(1)	90	123(1)	92	42(1)	10(2)														403	2.61	.38	14.6
	Lax						3	12	24	23	51(1)	57	66	58(2)	57(1)	42(1)	25(2)	4	3(1)				425	4.72	.73	15.5
(Quality×Jenkin)×Quality [a]	Club			7	56	91(2)	100(1)	55(2)	18(3)	7	5(1)	1(1)											340	2.82	.42	14.9
	Lax							8(2)	7(2)	20(3)	35(7)	39(6)	48(3)	37(2)	56(3)	45(6)	38(9)	34(9)	18(4)	9(2)	5	2(1)	1(1)	402	5.15	.71	13.8
(Quality×Jenkin)×Jenkin [a]	Club		17	44	96	48	65	40	18	7	3		1											339	2.61	.53	20.3
	Lax														2							1(1)		3			
(Jenkin×Hard Federation)×Jenkin	Club		1	36	70	69	70	71	38	22	9	1	1											388	2.85	.53	18.6
(Jenkin×Hard Federation)×Hard Federation	Club			7	11	50	128	125	54	13	3													391	3.05	.55	18.0
	Lax							4	5	12	22	33	100	68	70	32	13	3						362	4.88	.54	11.1
(Jenkin×Marquis)×Marquis [a]	Club			1	6	19	23	18	5	2														74	2.90	.37	12.8
	Lax									6	6	15	11	8	4		1(1)							51	4.55	.48	10.6

[a] Number of dwarfs in each frequency group is indicated by number in parenthesis.

for both the club and lax segregates so as to show the overlapping in density of the two classes which occurred in all crosses. Data on segregation for rachis density in the back cross (Quality × Little Club) × Quality, with those for the parents, are represented in Figure 2, A.

The mode of the Little Club parent is at 2.3 and that of Quality at 4.7. The mode of the *compactum* (club) group in the (Quality × Little Club) × Quality back-cross segregates is at 2.6, while that of the lax group at 4.7 corresponds with that of the Quality parent. The curve shows similar *compactum* and *vulgare* groups, indicating a 1:1 ratio. The lax group showed wide variation and extension beyond the limits of the lax parent, suggesting the presence of modifying factors for the lax character.

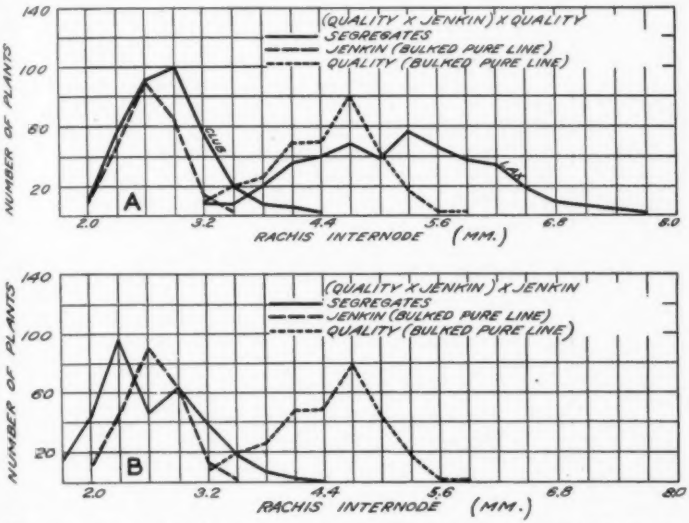

FIGURE 3.—Curves for spike density in wheat back crosses: A, (Quality × Jenkin) × Quality; B, (Quality × Jenkin) × Jenkin

The curve for the (Quality × Little Club) × Little Club (fig. 2, B) transgresses the range of that of the club parent. The measurements of the lax segregates of this cross were omitted in plotting the curve, as these segregates probably were produced from unsuccessfully back-crossed F_1 kernels. The broad flattened curve with the main peak at 2.6, which covers the mode of the Little Club parent, indicates segregation of club (*compactum*) and lax factors in the F_1 hybrids carrying the gametes.

The curves for the remaining three crosses, presented in Figures 3, 4, and 5, show similar relations between the club and lax groups, indicating a 1:1 ratio in each case. The curve for the (Quality × Jenkin) × Quality back cross (fig. 3, A) shows a preponderance of lax plants, probably resulting from the presence of dwarfs. The dwarfs will be discussed in this connection later. In the (Jenkin × Marquis) × Marquis back cross (fig. 5), on the other hand, there is an

excess of club plants. The tendency to a bimodal distribution of the lax segregates in (Jenkin × Hard Federation) × Hard Federation back-crossed population (fig. 4, A) probably is due to recombinations of minor factors for laxness. The curve for the (Quality × Jenkin) × Jenkin back cross (fig., 3 B) is bimodal, indicating segregation in the F_1 of club and lax factors in equal numbers. The curve for the (Jenkin × Hard Federation) × Jenkin back cross (fig. 4, B) is broadly flattened and slightly bimodal, indicating similar factor relations.

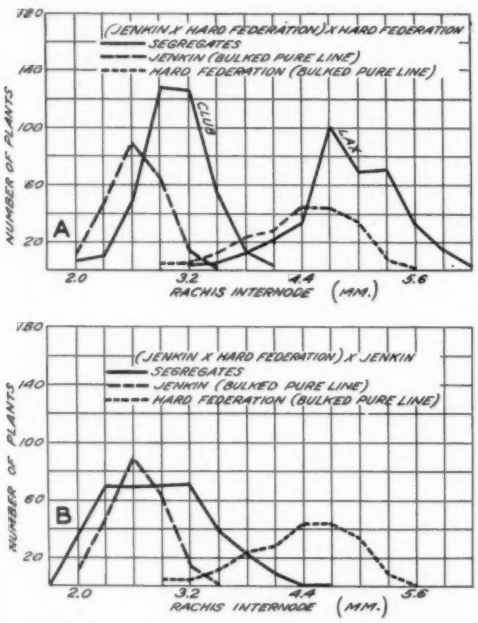

FIGURE 4.—Curves for spike density in wheat back crosses: A, (Jenkin Hard × Federation) × Hard Federation; B, (Jenkin × Hard Federation) × Jenkin

The numbers of club and lax plants in the back-crossed populations are summarized in Table 5. Statistical constants were calculated for the back crosses with the recessive (lax) parent. A close agreement to a 1 : 1 ratio was obtained in (Quality × Little Club) × Quality and in (Jenkin × Hard Federation) × Hard Federation. In (Quality × Jenkin) × Quality and in (Jenkin × Marquis) × Marquis the deviation was 3.4 and 3.0, respectively, times the probable error.

The lax plants in (Quality × LittleClub) × Little Club and (Quality × Jenkin) × Jenkin probably are due to selfed instead of back-crossed F_1 seeds. An entire uncrossed head probably was included by mistake in the first-mentioned back cross.

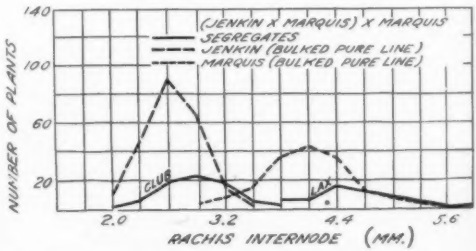

FIGURE 5.—Curves for spike density in wheat back cross (Jenkin × Marquis) × Marquis

TABLE 5.—*Club heads and lax heads as determined by inspection of common-wheat×club-wheat back crosses, 1927*

Back cross	Plants (including dwarfs)			Deviation from calculated 1:1	P. E.	Dev./P. E.
	Club	Lax	Total			
	Number	*Number*	*Number*			
(Quality×Little Club)×Little Club	351	13	364			
(Quality×Little Club)×Quality	403	425	828	11	9.7	1.1
(Quality×Jenkin)×Quality	340	402	742	31	9.2	3.4
(Quality×Jenkin)×Jenkin	339	3	342			
(Jenkin×Hard Federation)×Jenkin	389		389			
(Jenkin×Hard Federation)×Hard Federation	391	362	753	14.5	9.3	1.6
(Jenkin×Marquis)×Marquis	74	51	125	11.5	3.8	3.0

The spike studies showed that, because of the overlapping of frequency classes, classification of dense and lax types of spike by internode measurements is not always dependable. On the other hand, club and lax heads can usually be readily separated by inspection. For analysis of segregations for density in the club and lax groups, density measurements are essential.

SPIKE DENSITY IN F_2 GENERATION

Seed of the F_1 crop grown at Palo Alto in the summer of 1925 was sown at Davis in December of the same year. Two F_2 families, each from an F_1 plant, of each cross and its reciprocal were grown, except in the case of Jenkin×Quality and its reciprocal cross, of which only one family of each was grown; all of these families were studied for spike density in 1926. The principal spike of each plant was measured for rachis density. The ratios of *compactum* spikes to lax spikes in all families of each cross were similar, so that data are presented only for the single family of each cross carried into the F_3. Density-frequency distributions for four crosses, based on rachis-internode measurements, are shown in Table 6.

Classification of spike density by inspection indicates a single-facto difference in a 3:1 ratio in the crosses Quality×Little Club, Jenkin×Hard Federation, and Jenkin×Marquis. The deviation from the theoretical was not significant in any cross except that of Jenkin×Quality.

Curves for density of parents and F_2 are shown in Figure 6. Segregation into a large club group and a small lax group in all crosses indicates a simple unit-factor relationship, which also is indicated in the back crosses with the lax parents. It is evident from a comparison of the F_2 data (Table 6) with those for the parents (Table 4) that all crosses show transgressive segregation. The considerable deviation noted as occurring in the Jenkin×Quality cross, where a segregation of 2 club to 1 lax was found in one family, may be attributed to the disturbing influence of the factor or factors responsible for dwarfing. As dwarf plants with clublike spikes are few in number, it may be that whatever causes the appearance of dwarf plants also influences spike development.

TABLE 6.—*Frequency distribution of average rachis-internode lengths by 0.3 mm. classes of four F_2 common wheat × club wheat crosses, and parent varieties, 1926*

Cross, type of spike, and parent	Number having average rachis-internode lengths (0.3 mm. classes) indicated																			Plants	Rachis-internode length		
	1.4	1.7	2.0	2.3	2.6	2.9	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9	6.2	6.5	6.8	7.1		Mean	Standard deviation	Coefficient of variation
																					Number	*Mm.*	*Mm.*	
Quality×Little Club:																								
Club	5	20	41	47	26	9	4	1													153	2.23	0.40	17.9
Lax								2	10	20	17	4	5	1							59	4.25	.38	8.9
Little Club				4	2																6			
Quality									2	10	7	2									21	4.23	.24	5.7
Jenkin×Quality:																								
Club	1	22	23	38	33	23	9	5													154	2.41	.47	19.5
Lax				1	1		1	8	4	18	23	9	7	1	3	2					78	4.33	.63	14.6
Dwarfs—																								
Club					5	2	1	2													10			
Lax								3	2	2	2	1	4	3		1		1		3	22			
Jenkin					6	10	1														17	2.81	.18	6.4
Jenkin×Hard Federation:																								
Club	4	9	29	21	27	22	13	4	4												133	2.50	.55	22.0
Lax					1				4	12	15	2	2	4	1						41	4.37	.52	11.9
Hard Federation									4	13	6										23	4.13	.19	4.6
Jenkin×Marquis:																								
Club			6	10	16	26	22	17	3												100	2.93	.45	15.4
Lax									1	5	14	3	4	6	3	2					38	4.75	.55	11.6
Dwarfs—																								
Club								2													2			
Lax											1		3			1					5			
Marquis										2	7	6	4								19	4.59	.26	5.7

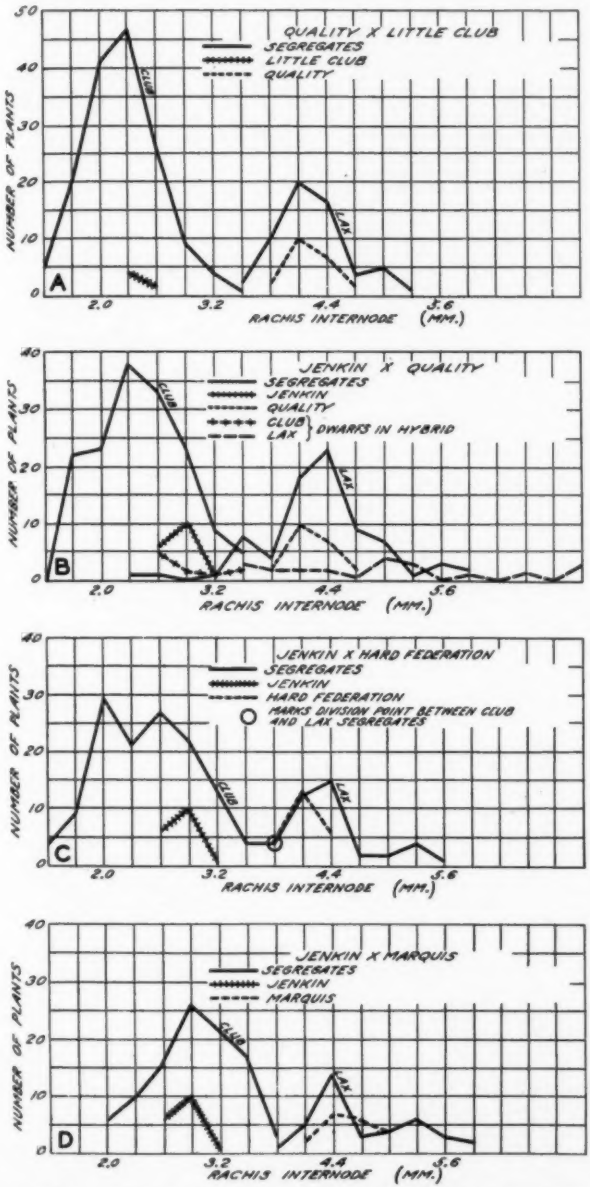

FIGURE 6.—Curves for spike density in F_2 of common-wheat×club-wheat crosses: A, Quality×Little Club; B Jenkin×Quality; C, Jenkin×Hard Federation; D Jenkin×Marquis

SPIKE DENSITY IN F_3 GENERATION

Curves were constructed from data on the F_2 families chosen for continuing the studies in the F_3. Plants were selected at random from various points on the curve covering the entire range of variation. The most numerous selections were made from the best plants of the club group and as many as possible from the limited number of extremely long lax heads.

The seeds from these F_2 plants were spaced about 1.6 inches apart in rod rows 1 foot apart. Each row contained from 50 to 120 seeds. Excellent stands were obtained. However, as a result of excessive winter moisture followed by drought and infection with foot rot, there was much inferior material. Spikes from foot-rot infected plants were poorly developed and more or less discolored. However, length of rachis on infected plants was reduced severely in only a comparatively small number of cases. A considerable number of plants were injured by the summer drought, as was shown by many undeveloped small heads. Diseased and drought-injured heads were eliminated as far as possible in the internode measurements, but all were counted in the inspection for density.

True-breeding families and club and lax segregates of varying densities occurred in the F_3 of the four crosses. The variations in density transgressed those of the parents. Such relations are considered positive evidence of modifying factors.

The F_3 data show that true-breeding club types can be obtained readily from an F_2 population if plants with very short, dense heads are chosen. Maximum density limits for club heads can not be given because of seasonal variation. However, true-breeding club families were obtained in all crosses from F_2 spikes having average rachis-internode lengths of 1.6 mm. or less. The effort to segregate the modifying factors in the lax group met with but little success, since the extremely long lax spikes chosen from the F_2 populations in most cases produced varying degrees of laxness. There appeared to be a preponderance of plants having long lax spikes in only one or two families.

Data on density of F_3 families are not presented in tabular form. The summarized F_3 data show that the ratio of club to lax heads was close to 3:1 in each case. The deviations which occurred can be accounted for by small populations and somewhat abnormal growing conditions.

DWARFING

Ordinarily the dwarf character is first apparent in the F_2 and later generations, but exceptions occur. Sax (*14*), in a cross of Bluestem × Amby, obtained grassy dwarf offspring in F_1. The writer obtained similar dwarf offspring in the F_1 of Canberra (C. I. 4986) × Marquis (C. I. 3641) at Davis, Calif., in 1922.[5] Leighty [5] found dwarfs in F_1 of crosses involving the soft red winter wheats Minhardi, Kawvale, and a selection from Reliable, at Sacaton, Ariz., in 1930. Others have found similar dwarfs in F_1 material.

Clark and Hooker (*3*) found a 2-factor difference for dwarfing in what was interpreted as a ratio of 13 normal to 3 dwarf plants in an F_2 of the cross Marquis × Hard Federation. They assumed a factor *DD* for dwarfs and an inhibitor *NN*, or factor for normal.

[5] Unpublished data.

Hayes and Aamodt (*9*) found 666 normal to 121 dwarf plants, or a ratio approaching 13:3 in the F_2 of Kota×Marquis. They interpreted their data on the basis of a 2-factor difference.

Waldron (*16*) also studied dwarfing in the cross Marquis×Kota and interpreted his results on a 3-factor difference. He assumed a dwarfing factor *D*, which, in the presence of an activating factor *A*, produced dwarfs in the absence of the normal factor *N*. The interaction of these factors would give a ratio of 55 normals to 9 dwarfs in the F_2.

Goulden (*7*) continued the study of dwarfing in F_3 of the Kota× Marquis material used by Hayes and Aamodt. He assumed, as did Clark and Hooker, a dominant factor *D* for dwarfing, which, in the absence of the inhibitor *N* for normal, produced dwarfs. His normal F_2 plants chosen at random produced true-breeding normal and segregating dwarf and normal families in the proportion 7:6, which favors the 2-factor hypothesis and the 13:3 ratio rather than the 3-factor hypothesis and the 55:9 ratio.

Clark and Quisenberry (*5*) likewise found a 2-factor difference in the cross Marquis×Kota and a very good fit in F_2 to a 13:3 ratio. Their F_3 data gave a very close fit to the expected proportion of seven true-breeding families to six families segregating for normals and dwarfs. These authors concluded that the genotype of Marquis was *ddnn* and of Kota *DDNN*.

DWARFS IN BACK CROSSES

A significant number of dwarf plants occurred in two of the crosses and back crosses studied, as already noted. This character appeared to modify to some extent the density ratios. The dwarf condition seems to be positively correlated with laxness of spike, since only a very few pure-club or intermediate-club dwarfs were found.

The distribution of club and lax plants among the dwarf segregates of four back crosses is shown in Table 7. In families having both club and lax plants there was a lower proportion of club than of lax plants, except in the (Quality×Jenkin)×Jenkin back cross. Here one of the two dwarfs was club, the other lax. In the (Quality× Jenkin)×Quality back-cross population there were six times as many lax as club plants, while in the (Jenkin×Marquis)×Marquis population there were 2 lax plants, no club plants, and 14 grassy plants. The large number of grassy plants (or plants without culms) in this population probably was due to the fact that lateness in maturing and the early dry season prevented the development of spikes.

TABLE 7.—*Distribution of club and lax plants among the dwarf segregates in four back crosses, 1927*

Back cross	Number of dwarfs—				
	Club	Lax	Unclassified	Grassy	Total
(Quality×Little Club)×Quality	4	8	2		14
(Quality×Jenkin)×Quality	14	84		4	102
(Quality×Jenkin)×Jenkin	1	1			2
(Jenkin×Marquis)×Marquis		2		14	16

The spikes of lax dwarfs usually were extremely lax, while those of club dwarfs usually were intermediate or long club. Dwarf plants of club type rarely occurred. Data on spike density of dwarfs occurring in the (Quality × Jenkin) × Quality population are presented in Table 4. The number of dwarfs for each frequency class is given in parentheses. Most of the lax dwarfs had an average rachis-internode length of over 5 mm.

The distribution of normal and dwarf plants in two back-crossed populations is given in Table 8. The ratios of normal to dwarf plants were 6.9:1.09 in (Quality × Jenkin) × Quality and 7.08:0.92 in (Jenkin × Marquis) × Marquis.

TABLE 8.—*Distribution of normal and dwarf plants in wheat back-crossed populations, 1927*

Back cross	Number of plants—			Deviation *	P. E.	Dev. / P. E.
	Normal	Dwarf	Total			
(Quality × Jenkin) × Quality	649	102	751	8.1	6.11	1.3
(Jenkin × Marquis) × Marquis	123	16	139	1.4	2.63	.5

* Deviation from calculated 7 : 1.

DWARFS IN F_2

Two hundred and thirty-three normal and 49 dwarf plants, or a ratio of 4.8:1, were found in an F_2 family of the cross Jenkin × Quality. If the data for this family are combined with those for the reciprocal family, the numbers are 569 normal to 89 dwarf, or a ratio of 6.4:1. There were 432 normal and 71 dwarf, or a ratio of 6.1:1 in five Jenkin × Marquis and reciprocal families. Segregation for dwarfs was not studied in the F_3, but the results obtained in the F_2 appear to give the best fit in the ratio of 55 normal to 9 dwarf. This would be a ratio of 6.1:1, which is exactly duplicated in the F_2 of Jenkin × Marquis when the individuals of all F_2 families, including reciprocals, are summated. Moreover, the ratio of 6.4:1 of the cross Jenkin × Quality does not deviate widely from the theoretical ratio 6.1:1.

The occurrence of similar ratios in both back crosses and of nearly the same ratios in the F_2 of the original crosses makes it seem likely that the factorial relations for dwarfing are similar in the two crosses.

GLUME COLOR IN THE BACK CROSS AND IN F_2

Glume color was studied in the (Jenkin × Marquis) × Marquis back-cross population. The glume color of the brown-glumed plants of this population was lighter in shade than the glume color of the Jenkin parent plants. The segregation in the back-cross generation was 58 brown to 50 white. This ratio of 1.2:1 indicates a single factor for glume color.

Two F_2 families of Jenkin × Marquis sown at the same time as the back crosses gave observed numbers of brown-chaffed to white-chaffed plants of 174:56 and 133:43, respectively, which, summated, gives 307:99 and an observed ratio of 3.01:0.99. This evidence for a 1-factor difference confirms that from the back cross and is in agreement with results obtained by numerous investigators.

Earliness

A number of workers have studied the inheritance of earliness in wheat hybrids. Their results indicate that earliness is governed by complex factor relations. In most cases they make no attempt to specify the number of factors involved. The writer (*6*), in a cross between Sunset, a very early variety, and Marquis, a late variety in California, obtained results indicating a primary unit-factor difference, with possibly several minor modifying factors.

Earliness in Back Crosses

Realizing the difficulty involved in determining factorial relations in the inheritance of earliness in the ordinary F_2 and F_3 generations, a study of this character was attempted in back-crossed material. Quality × Little Club and Quality × Jenkin were back crossed to both of their respective parents. In each of the resulting crosses the F_1 was intermediate in earliness, as may be seen in Table 9, which shows the data on earliness of parents and back crosses.

A comparison of the earliness of the bulked pure lines used in pollinations and of that of selections from the progeny of the original parents also was made. The range of variation in the bulked pure-line plants of both Jenkin and Little Club is nearly identical with that of their respective plant selections. A number of the bulked pure-line plants of Quality were about a week earlier than the earliest plants of the Quality selection 22–1, but the modes for the two distributions were identical. Since the bulked pure lines of all varieties were uniform in type, it is believed that the pure lines and their respective selections were genotypically identical.

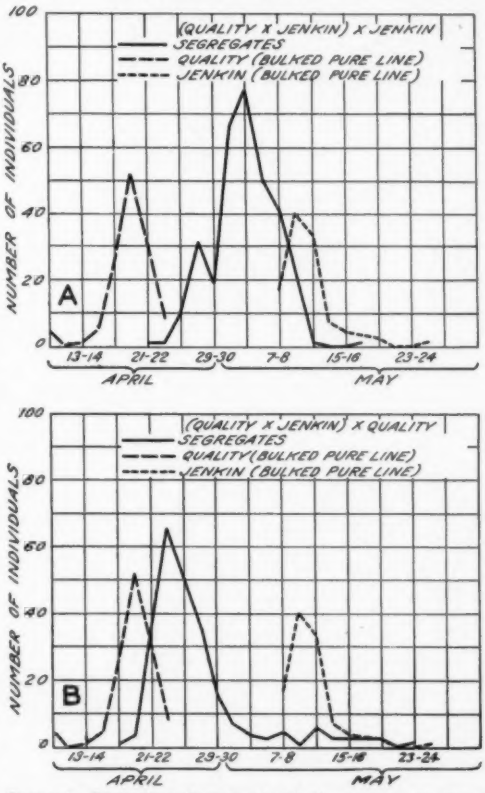

FIGURE 7—Curves for earliness in wheat back crosses: A, (Quality × Jenkin) × Jenkin; B, (Quality × Jenkin) × Quality

Curves for earliness in the four back crosses are shown in Figures 7 and 8. Figure 7, A, presents a comparison of the earliness of the

TABLE 9.—*Frequency distributions of dates of first heading of parent and back-cross populations, 1927*

Parent and back cross	Number headed on dates of first heading (2-day classes) given																								Total number of plants
	April												May												
	9–10	11–12	13–14	15–16	17–18	19–20	21–22	23–24	25–26	27–28	29–30	1–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17–18	19–20	21–22	23–24	25–26	
Quality	5		1	5	25	52	30	8																	126
Jenkin															17	40	34	7	4	3	2			1	108
Little Club													1	4	15	42	22	10	5	7					106
(Quality×Little Club) ×Little Club						1		4	13	27	23	40	61	55	34	22	9	6	3		1				299
(Quality×Little Club) ×Quality [a]					1	31	49	82	74	54	16	7 (1)		4 (1)	2 (2)	3 (2)	1 (1)								324
(Quality×Jenkin)× Quality [a]					1	4	37	65 (1)	51 (2)	36 (1)	15 (1)	7 (2)	4	3 (2)	5 (1)	1 (1)	6 (6)	3 (3)	3 (3)	3 (3)	3 (3)		1 (1)		248
(Quality×Jenkin)× Jenkin [a]							1	1	10	31	19	67	77	49	42	23	1			1 (1)					322

[a] Number of dwarfs in each frequency group is indicated by number in parenthesis.

(Quality × Jenkin) × Jenkin back-cross population with that of both parents. The back-crossed population might be expected to contain plants heading over the entire range of Jenkin, the late or recessive parent. As a matter of fact, the back-crossed population was intermediate but inclined to the late parent, and only partially covered the range of Jenkin. The curve is bimodal, having a small mode on the side toward that of the early parent.

In the back cross with Quality, the early parent, the curve (fig. 7, B) is unimodal and strongly inclined toward the early parent. The modes of the back cross and the early parent are separated by only two frequency classes, or four days. The range in the other direction extends almost completely over that of the late parent because of the dwarfs found in this population. Dwarf plants generally are late in maturing. About three-fourths of the dwarfs headed within the range of the Jenkin parent; the remainder were intermediate. None reached the mode of the early parent.

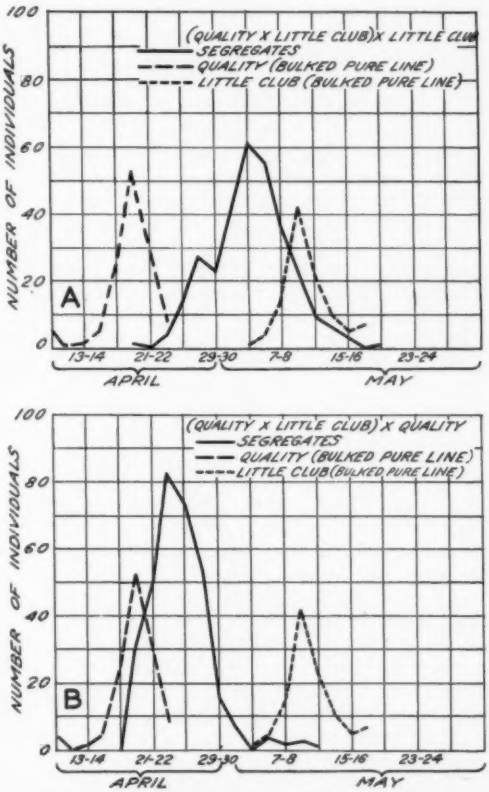

FIGURE 8.—Curves for earliness in wheat back crosses: A, (Quality × Little Club) × Little Club; B, (Quality × Little Club) × Quality

Figure 8, A, shows the curve for earliness in (Quality × Little Club) × Little Club. Here also the bulk of the population was intermediate between that of the parents, but its range entirely covers that of the late or recessive parent. The curve is bimodal, having a small mode on the side toward the early parent.

Figure 8, B, shows the curve for earliness in (Quality × Little Club) × Quality. The curve is unimodal and strongly inclined toward that of the early parent. The modes of the early parent and of the back cross, as in Figure 7, B, are separated by two frequency classes, or four days. The dwarf plants extended the range of earliness into that of the late parent.

DISCUSSION

As is well known, the basis for the use of the back cross in genetic analysis is the crossing of the F_1 hybrid of a cross with its recessive parent, which permits the recessive factors of the hybrid gametes to come to expression. When an F_1 hybrid having an independently segregating single-character difference is back crossed to the recessive parent, a 1:1 ratio of dominants to recessives is obtained in the F_1 of the back cross. With a 2 factor difference, four different kinds of gametes would be formed from the F_1, which, when back crossed to the double recessive, would give four equal classes of zygotes in a 1:1:1:1 ratio. As only one class would be pure recessive the dominant genotypes would be indistinguishable, and a 3:1 ratio would be obtained in the F_1 of the back cross. With three factors present, eight kinds of gametes would be formed. These, when back crossed to the triple recessive, would give eight equal classes of zygotes. As it would be likely that only the triple recessives could be distinguished with certainty, the F_1 of the back cross would give a ratio of 7:1.

In the back-cross method a smaller number of individuals is required to obtain reliable ratios than in an F_2 of a regular segregating hybrid generation. Ordinarily an F_2 generation of a back cross should be grown to verify the results obtained in the back-crossed F_1. However, evidence from the back cross, together with the results obtained from a regular F_2 generation, should give fairly satisfactory evidence of the correct factorial relations.

One disadvantage of the back-cross method is that all of the desired recessive characters are not always found together in one parent. In many plants the back-cross method is impracticable on account of the labor involved in making crosses.

The difficulty in making crosses has almost entirely prevented the use of the back-cross method in wheat breeding except for intensifying certain characters or qualities. Watkins (*17*), in his genetic and cytologic studies of wheat, made use of the back cross for factorial analysis in small families (about 30 or 40 plants) and advocated its use wherever possible. Where it is desired to study back crosses of wheat it is possible to obtain fairly large populations with moderate ease by the technic of hybridization used by the writer. As already mentioned, 4,692 apparently back-crossed kernels were obtained from 231 crossed heads. Approximately 50 hours were required by each of two workers to perform the necessary operations, which were accomplished on 14 different days.

Considerable parent material was required to provide pollen. As there was a marked difference in time of maturity in the two parents, sowings of the early parent were made on different dates. The individual parent seeds were spaced about 2½ inches apart in the row. Ten to twelve inches would have permitted the development of a larger number of culms. If possible, all the pollen required for a certain back cross should be procured from one plant.

SPIKE DENSITY

A 1-factor difference between the club and lax types of spike was found in the back crosses of the four varietal combinations studied by the writer. This was verified by results obtained in the regular F_2 and F_3 generations. The attempt to determine minor factor

differences in these generations was not successful. However, judging by the occurrence in certain F_3 families of both club and lax segregates of different densities, such minor factors evidently were present.

Two of the F_2 families segregated for dwarf plants, but no dwarfs occurred in the F_1. The majority of dwarf plants had lax spikes; most of the remainder were intermediate in type, while compact club dwarfs rarely occurred.

DWARFING

A 3-factor difference between the parent varieties is indicated by the F_2 ratio of 55 normal to 9 dwarf in the crosses Jenkin × Marquis and Jenkin × Quality. This segregation is explained by assuming the normal factor N and the complementary dwarfing factors D_1 and D_2, operative only in the absence of N. As both of these factors are necessary to produce dwarfs they are not identical with the factor D found in Kota.

The factorial constitution of the parent varieties with reference to dwarfing could be one of at least two different combinations of genotypes: (1) The N factor and both dwarfing factors may be present in one parent, $NND_1D_1D_2D_2$, and all of the recessive factors in the other parent, $nnd_1d_1d_2d_2$, or (2) the N factor, a dominant dwarfing factor, and one recessive factor may be present in one parent, $NND_1D_1d_2d_2$, and two recessive and the other dominant dwarfing factor in the other parent, $nnd_1d_1D_2D_2$. The latter, in the absence of N, would be normal. The F_1 back crossed to $nnd_1d_1D_2D_2$ would result in a progeny of 3 normal to 1 dwarf. To get a ratio of 7 normal to 1 dwarf it would be necessary to cross back to a triple recessive. Since a ratio closely approximating 7 normal to 1 dwarf was obtained in back crosses with both Quality and Marquis, both of these varieties should have the genotypic constitution $nnd_1d_1d_2d_2$, and that of Jenkin would be $NND_1D_1D_2D_2$.

According to the hypothesis stated, the F_1 of the crosses involving Marquis and Quality would have the genotypic constitution $NnD_1d_1D_2d_2$. The F_1 would produce different kinds of gametes, the recessive parent only one kind of gamete, nd_1d_2. The genotypes and phenotypes of the back-crossed generation are shown in Table 10.

TABLE 10.—*Genotypes and phenotypes of back-crossed F_1 hybrids between Marquis and Quality*

Genotype	Phenotype *	Frequency	Genotype	Phenotype *	Frequency
$NnD_1d_1D_2d_2$	Normal	1	$nnD_1d_1D_2d_2$	Dwarf	1
$NnD_1d_1d_2d_2$	do	1	$nnD_1d_1d_2d_2$	Normal	1
$Nnd_1d_1D_2d_2$	do	1	$nnd_1d_1D_2d_2$	do	1
$Nnd_1d_1d_2d_2$	do	1	$nnd_1d_1d_2d_2$	do	1

* Distribution, 7 normal to 1 dwarf.

Further evidence of the correct factor difference for dwarfing should be obtained by growing progeny of the dwarfs of the back-crossed generation. According to the hypothesis, these dwarfs, being of the genetic constitution $nnD_1d_1D_2d_2$, should produce prog-

eny in a ratio of 7 normal to 9 dwarf. Progenies from 21 dwarf back-crossed plants grown in 1929 produced 31 normal to 77 dwarf and semidwarf plants. The small size of families resulted from injury to seed caused by Angoumois grain moths while the seed was in storage at Davis in 1928. As all dwarf back-crossed plants should be of identical genetic constitution, the seed actually producing plants in the second generation of the back cross probably would represent a random sample and the segregation observed should be representative. Instead of the expected ratio of 7 normal to 9 dwarf, a ratio of about 1 normal to 3 dwarf was obtained.

The back-cross data demonstrate in a convincing way the genotypic constitution of the recessive parents as triple recessive. The presence of dwarf factors, however, might cause abnormal zygotic dwarf mortality under certain conditions which could easily result in error in the observed segregation. There is some evidence of this in the Kota × Marquis cross reported by Goulden (*7*), which produced a ratio of close to 55 normal to 9 dwarf. His data on the F_3, however, supported a 2-factor hypothesis and indicated a ratio of 13 normal to 3 dwarf in F_2.

It is also possible that the genetic constitution of Jenkin might be $NND_1D_1d_2d_2$ and that of Marquis $nnd_1d_1D_2D_2$. Marquis is known to possess one of two complementary factors for dwarfing, as shown by the fact that in certain of its crosses with normal varieties dwarf plants are produced in F_1. The cross Jenkin × Marquis would result in a ratio of 55 normal to 9 dwarf in F_2, but the back-cross dwarfs would produce a ratio of 1 normal to 3 dwarf in second-generation families.

Further experiments are needed to identify the type of factor differences for dwarfing involved in these crosses.

The preponderance of lax-spike plants among dwarfs suggests a linkage between one of the factors responsible for dwarfing and the factor for lax spikes. The normal allelomorph of the linked dwarfing factor would also be linked with the club factor *C*. Assuming this dwarfing factor to be D_1, then crossing over between the linked factors D_1C and d_1C of the heterozygote would give gametes having the factor combinations d_1c and D_1C. The latter, in proper combination with other factors, would give a club dwarf.

With close linkage very few dense or pure-club dwarfs would be produced, the chances for such recombinations being slight on account of the limited number of crossover club gametes. Dwarfness in wheat and other cereal plants is considered a semilethal condition and it may be possible that the homozygous dwarf condition is lethal to a certain extent. However, no such lethal plants have been observed.

EARLINESS

It is evident from the nature of the curves shown in Figures 7 and 8 that in the crosses studied earliness depends on more than one factor. If due to a 1-factor difference, the curves should be bimodal in two approximately equal groups. But this is not the case. In the curves obtained, a small mode, probably not significant genetically, occurs in both crosses on the side toward the early parent. These small modes are exactly intermediate between those of the parents, while the bulk of the population is inclined toward the late parent.

A study of the figures shows that back crossing with both parents demonstrates that both parents contribute dominant factors for earliness. If one parent contained all the recessive factors, the recessive segregates would be grouped around the mode of the recessive parent. It might be possible to determine the factor relations for earliness by the relative amount of shift of the modes of the back-crossed populations from the modes of the parents.

Three or more factors probably are responsible for the difference in earliness observed between Quality and Jenkin and Quality and Little Club.

SUMMARY

Two leading club (*compactum*) wheats were crossed with common (*vulgare*) wheats of high quality to improve the milling qualities of the club wheats. The F_1 of these crosses was back crossed to facilitate selection of both "quality" factors and club types.

This material was utilized to make a study of inheritance of characters in the F_1 of the back-crossed generation and a collateral study of inheritance of the same characters in the ordinary F_2 and F_3 generations. Back crosses were made to both parents in Quality × Little Club, Quality × Jenkin, and Jenkin × Hard Federation; and to the Marquis parent in Jenkin × Marquis. Spike density was studied by means of rachis-internode measurements and by inspection in all crosses; dwarfing, in Quality × Jenkin, and in Jenkin × Marquis; color of glume, in Jenkin × Marquis; and earliness, in the back-crossed generation only in Quality × Jenkin and in Jenkin × Little Club.

The F_1 generation of these crosses was club, or *compactum*, in type and was designated as "long club."

A ratio of approximately 1 dense to 1 lax plant was obtained in all back crosses with the lax parent, except in (Jenkin × Marquis) × Marquis, where nearly all dwarf plants failed to head. A 1-factor difference for club type and lax type of spike was indicated.

These results were corroborated by those obtained in the collateral study of the regular F_2 and F_3 generations.

True-breeding club lines of varying density were recovered in the F_3, but no true-breeding long club like the Jenkin parent was found. Lax plants bred true, but most of the extremely long lax forms reverted to the approximate internode density of the lax parents. In two families a majority of long lax forms appeared. Variations in density in pure-breeding F_3 material indicate the presence of modifying factors for both club and lax characters.

Dwarfs occurred in the back crosses (Quality × Jenkin) × Quality and (Jenkin × Marquis) × Marquis, in a ratio of approximately 7 normal to 1 dwarf in each case. A ratio of about 55 normal to 9 dwarf occurred in the F_2 of both Quality × Jenkin and Jenkin × Marquis. Segregations in the back-crossed generation and in the F_2 of these two crosses indicate a 3-factor difference between normal and dwarf. Jenkin is assumed to have the constitution $NND_1D_1D_2D_2$ and Quality and Marquis $nnd_1d_1d_2d_2$. Additional evidence is needed to verify this assumption.

Most dwarf plants had lax spikes. Club dwarfs had intermediate club spikes in most cases. Dense club-spike dwarfs occurred rarely. The evidence indicates possible linkage between the club factor and one of the dwarfing factors.

In the (Jenkin × Marquis) × Marquis back cross the ratio of brown-glumed to white-glumed plants was 1.2:1, or approximately 1:1. A ratio of 3 brown-glumed to 1 white-glumed plant was obtained in F_2 of Jenkin × Marquis. A 1-factor difference for glume color is indicated.

Probably three or more factors are responsible for the difference in earliness observed between Quality and Jenkin and Quality and Little Club.

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VIABILITY OF STRAND HYPHAE OF THE COTTON ROOT-ROT FUNGUS [1]

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INTRODUCTION

Since the discovery of a sclerotium stage of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, by King and Loomis [2] in Arizona and by the senior writer [3] in Texas, the writers have endeavored to ascertain to what extent, if any, other stages of this pathogene may be significant in perpetuating the disease in the soil in Texas. During the season of 1929–30 studies of the viability of the conidial stage and of the subterranean strands of the fungus were made at the United States Cotton Breeding Field Station, Greenville, Tex., and at other localities in Texas.

STRANDS ASSOCIATED WITH THE CONIDIAL STAGE

Conidial mats were found in abundance during the latter part of April in an alfalfa field at Lone Oak, Tex., and in many cases they had reached a diameter of several centimeters, producing vast quantities of conidia. They were growing both on the surface and in deep crevices in the soil along margins of an active zone of infection. Several of these mats were placed in small paper boxes and removed to the laboratory for viability studies. In most cases a layer of soil (Wilson clay) an inch or more in thickness, which contained the underlying coarse strands of the fungus, was left attached to the mats. After removal to the laboratory the material was allowed to air-dry at ordinary room temperature for six and one-half months, and following this interval it was placed between filter paper in moist chambers and incubated at approximately 26° C.

Microscopic examinations were made at the end of 24-hour and 48-hour periods of incubation, and in the majority of cases new growth of the fungus occurred. (Fig. 1.) No actual germination of the conidia was observed at this time, but the rhizomorphic strand hyphae pervading the soil mass, directly beneath the spore mat, were viable in many cases.

The new growth of the fungus was vigorous and hyaline, and when examined under the microscope it appeared to originate from the ends of the broken strands. The large central cell of the strand hyphae, which is surrounded by three or four layers of somewhat similar but smaller cells, appeared to be the source of the new growth.

[1] Received for publication Mar. 24, 1931; issued October, 1931.

[2] KING, C. J., and LOOMIS, H. F. FURTHER STUDIES OF COTTON ROOT ROT IN ARIZONA, WITH A DESCRIPTION OF A SCLEROTIUM STAGE OF THE FUNGUS. Jour. Agr. Research 39: 641–676, illus. 1929.

[3] NEAL, D. C. THE OCCURRENCE OF VIABLE COTTON ROOT-ROT SCLEROTIA IN NATURE. Science (n. s.) 70: 409–410. 1929.

Following this discovery, a second visit was made on November 9 to this particular field, and other mats were collected as described above. These mats, however, were not found on the surface, but on the sides of deep crevices in the soil resulting from prolonged dry weather. The mats had doubtless formed two months previously during a period of wet weather. They were also placed in moist chambers, incubated as described above, and examined microscopically.

The same new-growth character of the fungus was again observed, but was somewhat more abundant, with the fresh mycelium appearing in small white tufts or patches on top of and beneath the spore mass. Exudation of the characteristic brown, viscous liquid, as frequently observed in cultures of *Phymatotrichum omnivorum* when in an active state of growth, was found at the ends of the strands.

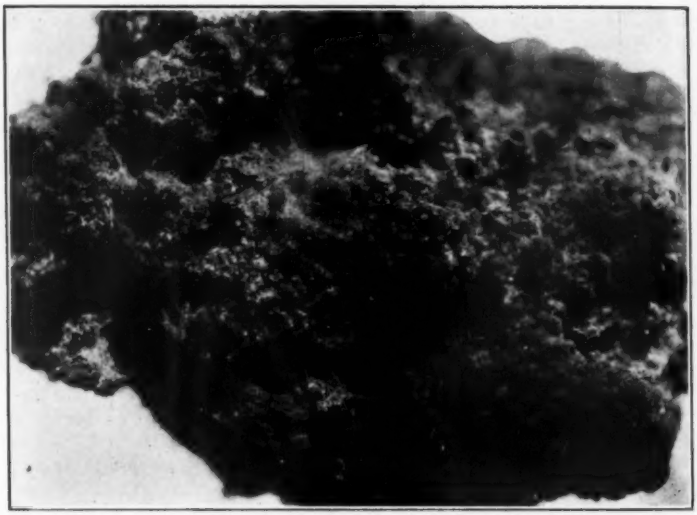

FIGURE 1.—Portion of soil including conidial development of *Phymatotrichum omnivorum*, air-dried for six and one-half months in open boxes in the laboratory, showing new growth of strand hyphae after 48 hours' incubation in a moist chamber. × 7

A few days later, growth of the mycelium was noticeably increased, with other branches appearing and possessing the characteristic plectenchymatic bands of irregularly shaped cells typical of strand hyphae, together with well-differentiated radiating acicular branches.

Cultures were not obtained from the fresh growth of the fungus in the mats for infection experiments, but weed and alfalfa seed, which had become lodged in the desiccated soil masses, germinated when placed in the moist chambers, and later the seedlings were killed by the growing fungus.

With the exception of the mats collected in the alfalfa fields, the writers have not had the opportunity to study this unusual behavior of viability of strands associated with conidial formation on either cotton or other susceptible hosts, largely because the mats occur rather infrequently in the infested areas under observation.

STRANDS OCCURRING IN THE SOIL

Duggar,[4] in describing the strand hyphae occurring on roots of infected plants and in the soil, makes the following statement: "They are also more or less sclerotial and doubtless are an important factor in the persistence of the fungus in the soil." Dana[5] reports investigations connecting the appearance of root rot with strands of the fungus found in the soil as the source of infection, and Ratliffe[6] was able to obtain a new growth of the fungus from strands found in excavations beneath spore mats. However, these reports are based on observations made in July, and the strands obtained may have originated from sclerotia formed in the soil the preceding season. The writers conducted similar experiments, but delayed the tests for strand viability until December 10, at which time the plants were dead, the roots in most cases decomposed, and the fungus lying dormant in a sclerotial stage, as evidenced in several excavations. Strands were taken from the soil at various depths in an infested cotton plot which two years previously had been planted to grain. They were removed to the laboratory in Petri dishes, washed thoroughly in distilled water to remove the soil particles, and plated on corn-meal agar. At the end of a 24-hour period fresh mycelium was observed growing from the ends and to some extent at the sides of the broken strands.

The subterranean strands having been recovered and their viability established on the above date, excavations were made later in the season to study further their longevity. Numerous soil examinations were made at various depths during January, February, and March in the area previously examined and in several other infested cotton fields, but in every instance they failed to reveal the presence of the fungus in the soil. These data indicate, therefore, that the subterranean strands of the fungus, although partly comparable to sclerotia in cellular structure, do not persist in the soil as an overwintering stage.

DISCUSSION

The revival of growth of the fungus strands associated with the conidial stage is of interest in view of the fact that those found at different depths in the soil on infected roots succumbed readily when subjected to desiccation comparable to that given spore mats. However, the marked viability of the fruiting strands of the fungus is perhaps of small importance, since this stage of the disease, as previously mentioned, occurs rarely in infested cotton fields in Texas; yet they may be a factor in perpetuating root rot in alfalfa fields, where they frequently form in abundance during the spring and fall.

The writers have also observed that the strands of the fungus found on infected cotton roots and other plants do not maintain their viability for long periods under field conditions, as repeated efforts to use them as inoculum in both greenhouse and field experiments or for isolating the fungus have met with failure. In cultures their

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growth and virulence also become noticeably attenuated after a few weeks. In this connection, King and Loomis[7] state that they "have never been able to obtain growth from detached fragments of strands from the soil, nor from those entirely separated from the surface of infected roots." These strands apparently disintegrate in the soil during the winter, as evidenced in the excavations reported by the writers.

The occurrence of viable sclerotia in the soil,[8] however, and their connection with primary centers of infection in cotton fields in Texas reveal that the sclerotial stage of the fungus is largely responsible for the persistence of the disease.

SUMMARY

Viability studies of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, in the conidial stage and with subterranean strands, are reported.

Although no mycelial growth resulted from conidial germination, the strands permeating the spore mats maintained their viability after prolonged periods of drying in open boxes in the laboratory, but their rather infrequent occurrence in cotton fields in Texas suggests that they play a negligible rôle in the overwintering of the disease.

In the experiments reported, strands of the fungus recovered at various depths from infested cotton fields proved viable as late as December 10, but excavations made during January, February, and March failed to reveal their presence, thus tending to eliminate them as an overwintering possibility.

The negative results obtained with strands indicate that the sclerotium is the important stage of the pathogene concerned in maintaining its viability from year to year.

[7] KING, C. J., and LOOMIS, H. F. Op. cit. (See footnote 2.)

[8] NEAL, D. C. Op cit. (See footnote 3.)

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INFLUENCE OF HUMIDITY ON FLORAL INFECTION OF WHEAT AND BARLEY BY LOOSE SMUT[1]

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INTRODUCTION

In a study of the relative resistance of some wheat varieties to loose smut (*Ustilago tritici* (Pers.) Rostr.), previously reported by the writer (*12*),[3] the three club wheats Hybrid 128 (C. I.[4] 4229), Jenkin (C. I. 5177), and Little Club (C. I. 4066) proved strikingly susceptible when grown from seed from flowers that had been artificially inoculated under humid conditions. In view of the fact that loose smut occurs but rarely in the Pacific Coast States (fig. 1),

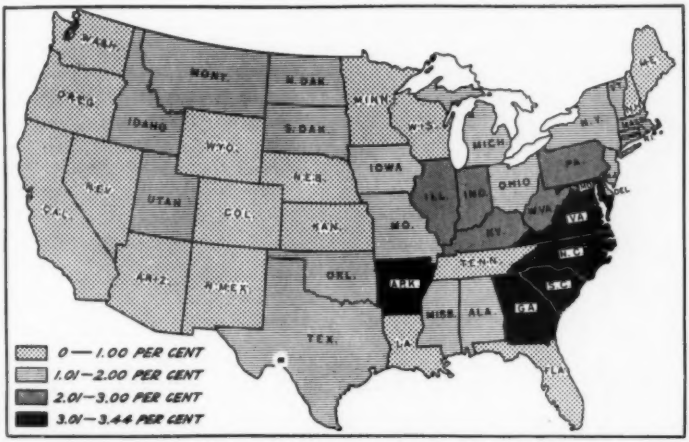

FIGURE 1.—Estimated average annual reduction of wheat production (per cent) due to loose smut in each State for the period 1917–1927. The average reduction (per cent) for each State has been placed in one of four groups. Data compiled from estimates of the Plant Disease Bulletin (footnote 6)

where these wheats are grown, it seemed probable that the low atmospheric humidity, which generally prevails there when the wheats are in bloom (fig. 2), might play an important rôle in preventing infection. The probability of this relation was strengthened by the writer's observations during the summers of 1928 and 1929 in the State of Idaho on the loose-smut content in wheats grown on

[1] Received for publication Apr. 6, 1931; issued October, 1931.
[2] The writer wishes to acknowledge his indebtedness to O. E. Baker, Bureau of Agricultural Economics, and A. G. Johnson, H. B. Humphrey, and C. E. Leighty, Bureau of Plant Industry for helpful suggestions, and to P. C. Day and W. W. Reed, Weather Bureau, U. S. Department of Agriculture, for the maps shown in Figure 2.
[3] Reference is made by number (italic) to Literature Cited, p. 515.
[4] C. I. indicates accession number of the Division of Cereal Crops and Diseases.

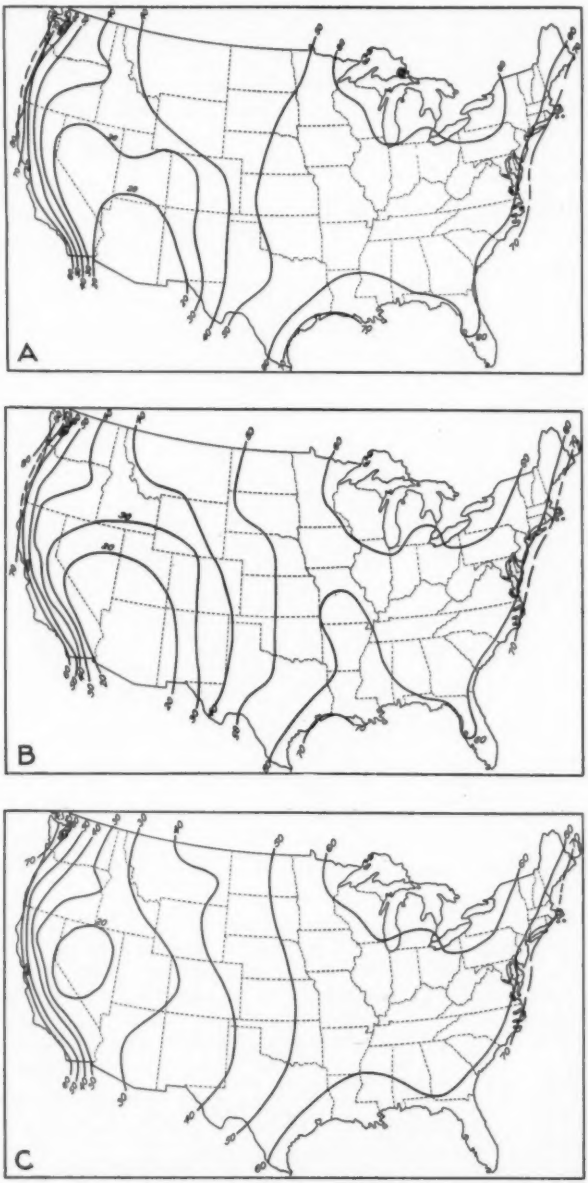

FIGURE 2.—Averages of relative humidity in percentages at 12 m., local time, for May (A), June (B), and July (C), for the 10-year period 1918–1927. Data from Weather Bureau

irrigated and on dry land. Loose smut was found prevalent and causing considerable damage in the wheats grown under irrigation but was seldom observed in the wheats grown on dry land. In this latter group the Turkey variety predominates. Three different selections of this wheat proved highly susceptible to loose smut in tests conducted by the writer (*12*).

In a study of the occurrence of *Ustilago tritici* in the United States, it was noted, furthermore, that this smut is more prevalent in the eastern than in the western half of the country.[5,6] (Fig. 1.) In this connection it is important to note that at the different periods when the wheats are in bloom [7] in the different sections in the United States the relative humidity, in general, also is higher in the eastern than in the western half of the country. (Fig. 2.)

In the United States, measures for controlling loose smut of wheat are rarely applied. Control through seed treatment is seldom employed, largely because the causal fungus, hibernating inside the seed, is not amenable to control by the easily applied surface disinfectants such as copper carbonate and formaldehyde. Treatments that thoroughly soak the seed, like the modified hot-water method, are necessary to kill the intraseminal mycelium. However, treatments of this kind are difficult to apply under the usual farm conditions. Furthermore, the modified hot-water treatment may cause severe injury to germination, thus reducing the yield (*11*). Recent studies by the writer (*12*) have shown that at least in the case of wheats grown in the Eastern States only a few commercial varieties are resistant. As a result of this situation the losses from loose smut in wheat continue unchecked.

It seemed desirable, therefore, to determine whether or not low relative humidity during the period of blooming might be the factor involved in the natural control of *Ustilago tritici*. It was felt that if a positive relation were found it might lead to a consideration of the practicability of control through the use of seed from areas with appropriately low ranges of relative humidity during the blooming period of the wheats.

The loose-smut fungus of barley (*Ustilago nuda* (Jens.) Kell. and Sw.) was also included in some of the studies herein reported. In general, the situation regarding the geographical occurrence of *U. nuda* and the hot-water treatment of the seed recommended for its control is similar to that of the loose-smut fungus of wheat.

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[5] In Idaho and Utah a considerable portion of the wheat is grown under the humid conditions accompanying irrigation, thus masking the effects of the normally low humidity on the control of wheat loose smut.

[6] UNITED STATES DEPARTMENT OF AGRICULTURE. BUREAU OF PLANT INDUSTRY. The following mimeographed material from Plant Disease Bulletin (or Reporter):

ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES. 1917. Plant Disease Bul. 2 (1), 18 p. 1918.
CROP LOSSES FROM PLANT DISEASES. 1918. Sup. 6, p. 186–213. 1919.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1919. Sup. 12, p. 307–332. 1920.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1920 Sup. 18, p. 317–338. 1921.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1921. Sup. 24, p. 489–510. 1922.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1922. Plant Disease Rptr., Sup. 30, p. 462–490. 1923.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1923. Sup. 36, p. 318–348. 1924.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1924. Sup. 43, p. 381–410. 1925.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1925. Sup. 49, p. 382–412. 1926.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1926. Sup. 56, p. 394–423. 1927.
CROP LOSSES FROM PLANT DISEASES IN THE UNITED STATES IN 1927. Sup. 64, p. 370–399. 1928.

[7] The approximate dates of blooming may be obtained from the harvest dates given (*2, 3*) by subtracting one month from the harvest dates.

LIFE HISTORY OF THE CAUSAL ORGANISMS

The fungus (*Ustilago tritici*) causing loose smut in wheat normally hibernates as mycelium within the wheat seed, vegetates systemically, and sporulates in the young wheat heads previous to their emergence, destroying most of the tissues of the spike, except the rachis, and producing a black powdery mass of smut spores in their stead. Smutted and healthy heads emerge during approximately the same period, and spores from the former are disseminated through the air while the healthy heads are in bloom. Inoculation occurs through the chance falling of one or more spores on the ovary or stigma, which is exposed through the opening of the glumes in the process of blooming. Within a few days all the spores are blown or washed from the diseased heads, and only the naked rachises of the spikes persist. Following their inclusion within the glumes of the healthy heads, the spores soon germinate. The germ tubes eventually enter the developing kernel and produce a mycelium, which hibernates within the matured kernel, thus completing the life cycle.

According to Freeman and Johnson (*6*), the life history of the loose-smut fungus of barley is similar to that of the loose-smut fungus of wheat. Like the latter, it infects its host only through the open flower and hibernates within the kernel as mycelium. To render the fungus innocuous it is necessary to apply a treatment like the modified hot-water method, which thoroughly soaks the seed. In recent years, however, Tisdale and Tapke (*13*) have produced loose smut in certain barleys as a result of seedling infection from smut spores applied to the mature seed, and Tisdale et al. (*14*), Tisdale and Tapke (*13*), Kirby (*7*), and others have noted that there is a varietal difference in the behavior of barleys toward seed treatment for the control of loose smut. Treatment of the seed with formaldehyde and certain organic mercury compounds has given partial or effective control in some varieties. Pending further addition to a knowledge of the factors involved in these new developments, the writer's studies reported herein have been confined to the floral infection of barley described by Freeman and Johnson (*6*).

PREVIOUS INVESTIGATIONS

Apparently, no previous investigations have been undertaken specifically to determine the rôle of humidity either in the infection, distribution, or control of the loose-smut fungi of wheat and barley, or any of the other cereals. However, several observations have been reported that have a bearing on the subject.

Mackie (*10, p. 618*) notes that "in California the dry season is near at hand when cereal plants are heading. Usually no considerable quantity of rain falls after this time," and that (*9, p. 2*) "the damage from loose smuts in wheat, barley, and oats in California is so slight that these smuts are not considered of economic importance." In studies on the floral-infecting loose smut of oats in Germany, Diehl (*5*) observed that smut spores alighting on the ovary of the blossoming flower germinate at once, except in very dry years.

PRESENT INVESTIGATIONS

EXPERIMENTS IN 1923–24

In the previous studies on the relative resistance of different wheat varieties, mostly eastern varieties, to loose smut (*12*), the writer included in the test in 1924 the three club varieties Hybrid 128 (C. I. 4229), Jenkin (C. I. 5177), and Little Club (C. I. 4066). These wheats are widely grown in one or more of the Pacific Coast States (*4*) where loose smut is very uncommon. (Fig. 1.) Smut-free plants from seed treated by the modified hot-water method were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in 1923. Flowers on these plants were inoculated by gently placing, with forceps, a small quantity of loose-smut spores on the stigmas of flowers in the process of blooming. The seed infected from such inoculation was harvested at maturity in March, 1923, and sown in a greenhouse the following autumn. The plants from this seeding were harvested in February, 1924, when fully headed. The number of plants and heads and the percentage of smutted plants and heads were obtained. The data are presented in Table 1.

TABLE 1.—*Percentages of loose smut in three varieties of club wheat grown in the greenhouse from seed from artificially inoculated flowers, Arlington Experiment Farm, Rosslyn, Va., 1923–24*

Variety	C. I. No.	Seeds sown	Plants matured		Plants smutted		Heads		
							Total	Smutted	
		Number	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Hybrid 128	4229	170	163	95.88	152	93.25	528	473	89.58
Jenkin	5177	140	138	98.57	134	97.10	463	452	97.62
Little Club	4066	175	160	91.42	156	97.50	651	632	97.08

Table 1 shows that the three club wheats, which rarely become smutted when grown in their habitat in the United States, were extremely susceptible when inoculated under the humid conditions prevailing in the greenhouse.

EXPERIMENTS IN 1927–28

In view of the suggestiveness of the data obtained in the foregoing preliminary experiment, the investigation was continued to determine definitely whether or not loose smut in wheat might be reduced or controlled by subjecting inoculated flowers to the low relative humidities generally prevalent during the blooming period of wheat in those parts of the United States where loose smut is uncommon.

MATERIALS, METHODS, AND RESULTS

Plants of a pure-line selection of Little Club (C. I. 4066) wheat were grown in 4-inch pots in a greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1927. At blooming, flowers were inoculated with freshly matured spores of *Ustilago tritici* from Little Club grown in another greenhouse. The technic of inoculation previously noted (p. 507) was again employed. Plants were inoculated on May 10, 1927, and placed under either arid or humid conditions for a period of eight days after inoculation. The plants subjected to humid conditions

were placed in a greenhouse in which the relative humidity varied between 56 and 85 per cent. The greenhouse floor was sprinkled from time to time to maintain a high humidity. Following this 8-day period, the plants were left to mature under the usual greenhouse conditions.

The arid condition was simulated by placing inoculated heads inside an elevated chamber containing air with a relative humidity reduced to 11 to 30 per cent through the use of calcium chloride. (Fig. 3.) The chamber was elevated so that the bottom was a few inches lower than the base of the heads of the shortest wheat plants.

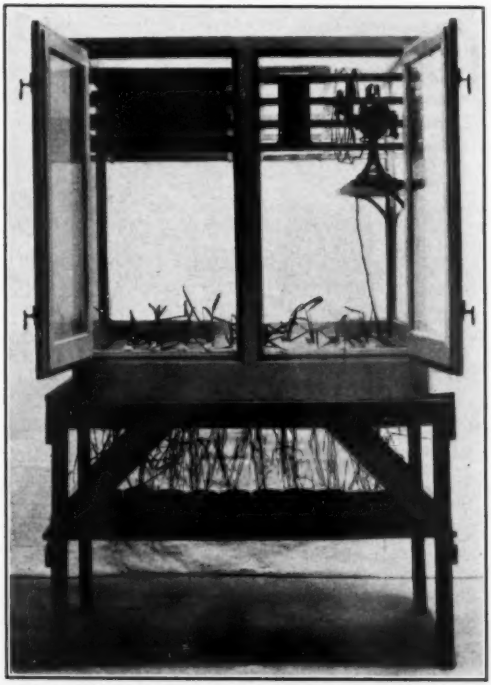

FIGURE 3.—Chamber used for subjecting wheat flowers inoculated with loose smut to low humidity

The bottom of the chamber contained numerous holes, seven-eighths of an inch in diameter, tightly plugged with corks. In order to introduce an inoculated head into the chamber, an opening was made by removing a cork. The head of wheat was inserted through the hole, as the plant bearing it was placed on the floor beneath the chamber. A ring of absorbent cotton was then tightly wound around the culm, at and just below the point of its passage through the bottom of the chamber, and tightly wadded into the hole. By this method inoculated heads were placed inside the chamber without noticeable effect on the moisture content of the contained air.

A low humidity was maintained by suspending 12 pounds of calcium chloride in shallow pans on racks near the roof of the chamber. An electric fan placed at one end of the pans circulated the contained air over the chemical. Two lots of calcium chloride were used. Each day one lot was removed from the chamber and replaced by the other, previously heated for three hours at 100° C. in a gas oven. The exchange was made at 10 a. m. daily. Following this operation, the relative humidity inside the chamber went down to 11 per cent within an hour, increasing to 30 per cent as the drying material became less absorptive.

Relative humidity was determined by a hygrometer placed in front of an electric fan as shown in Figure 3. The hygrometer was inserted, left 20 minutes, and read. It was then removed so as to influence the relative humidity inside the chamber as little as possible.

Flowering heads of Little Club (C.I.4066) wheat were inoculated by hand with freshly matured chlamydospores of *Ustilago tritici* and then left for eight days either in a humid greenhouse or in the low-humidity chamber. Each day during incubation a record of the humidity and temperature was made at 9 a. m., 1 p. m., 3 p. m., and 5 p. m. Relative-humidity percentages in the arid and humid groups ranged from 11 to 30 per cent and from 56 to 85 per cent, respectively. Temperatures ranged from 70° to 92° and from 67° to 84° F., respectively. The low-humidity chamber was protected from the direct rays of the sun, but, nevertheless, on bright days it was warmer than the humid greenhouse. The plants were removed from the low-humidity chamber on May 18. Plants of both groups were then placed together in the greenhouse and left to mature. According to Freeman and Johnson (*6*), wheat becomes increasingly resistant to infection as the period after blooming lengthens, losing its susceptibility entirely by the time the ovaries are one-third developed. At the end of eight days the ovaries of the inoculated flowers were more than one-third of their mature size and had passed the stage of susceptibility to infection, regardless of subsequent changes in environment. Any inoculum remaining in the flowers after the 8-day period, therefore, was incapable of producing infection. Seedling infection of wheat by *U. tritici* resulting from spores on the mature seed apparently has never been obtained. The 8-day postinoculation period also provided ample time for the fungus to reach its ultimate objective, the cavity of the ovary. According to Lang (*8*), this is normally accomplished in about one week after inoculation.

At maturity the kernels, developed in the inoculated flowers, were harvested and stored until the following winter (1927–28), when they were sown in the greenhouse. The resulting crop was harvested at maturity in the early spring of 1928. The results are presented in Table 2 and illustrated in Figures 4 and 5.

TABLE 2.—*Percentages of loose smut in Little Club wheat (C. I. 4066), grown in the greenhouse from seed from flowers incubated under the given conditions of humidity and temperature for eight days after artificial inoculation, Arlington Experiment Farm, Rosslyn, Va., 1927–28*

Atmospheric conditions during incubation		Seeds sown	Plants matured		Plants smutted		Heads		
Range in relative humidity	Range in temperature						Total	Smutted	
Per cent	*° F.*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
56–85	67–84	140	107	76.43	102	95.33	182	171	93.96
11–30	70–92	275	199	72.36	43	21.61	315	69	21.90

FIGURE 4.—Loose smut in Little Club wheat grown from seed from inoculated flowers subjected to a range of relative humidity of 56 to 85 per cent for eight days after inoculation. A, healthy plants; B, smutted plants

Table 2 and Figures 4 and 5 show a striking reduction in smut infection when inoculated flowers were subjected to ranges of low relative humidity and temperature similar to those occurring in the areas of the United States free from loose smut (fig. 1) during the blossoming period of wheat.

The influence of the difference in the ranges of temperature in this experiment can hardly be evaluated. According to Appel and Riehm (*1*), the cardinal temperatures for the germination of loose-smut spores are 42.8° to 50°, 80.6° and 91.4° to 93.2° F. Spores also were found capable of germination after an exposure of 12 hours to a temperature

of 96.8° In this experiment the temperatures were all within the germination range. It seems likely, therefore, that differences in humidity probably were accountable for most of the difference in loose-smut infection.

After the inoculations on May 10, several inoculated flowers were taken each day from plants in each of the two groups and examined microscopically. In the group held under humid conditions many spores were in the early stages of germination one day after inoculation. On the second and third days after inoculation approximately 90 per cent of the spores had germinated and produced long germ tubes intermingled with and ramifying the stigmas. The germina-

FIGURE 5.—Loose smut in Little Club wheat grown from seed from inoculated flowers subjected to a range of relative humidity of 11 to 30 per cent for eight days after inoculation. A, healthy plants; B, smutted plants

tion of the spores in flowers of the group held at low humidities presented a wholly different situation. Spores in the early stages of germination were not found until the third day after inoculation, and these were few in number. In later examinations, up to and including the time when the inoculated heads were removed from the dry chamber, only a few spores were found with germ tubes as long as those produced by spores in the other group two and three days after inoculation. These observations would seem to indicate that in the presence of low humidity loose smut in wheat is held in check because the smut spores either fail to germinate or germinate too slowly to enable the infecting hyphae to reach the ovary during the short period in which it is vulnerable.

EXPERIMENTS IN 1928–29

The study was continued at the Aberdeen substation, Aberdeen, Idaho, in 1928. In this year the experiments included the loose smut of barley in addition to the loose smut of wheat. Humid conditions were obtained by covering and spraying plants growing on irrigated land. Arid conditions were obtained by placing plants, in containers, in the desert and exposing them to the normally dry air of this arid region.

MATERIALS, METHODS, AND RESULTS

Federation (C. I. 4734) wheat and Moultan (C. I. 3401) and Hanna (C. I. 203) barleys were used. Federation wheat and Hanna barley were inoculated at the beginning of the second week in July, and Moultan barley was inoculated at the end of the third week in July. The technic of inoculation described on page 507 was employed. The inoculum used on Federation wheat consisted of chlamydospores of *Ustilago tritici* collected from the same variety a few days previous to inoculation. The smut used to inoculate the barleys consisted of a mixture of chlamydospores of *U. nuda* collected shortly before inoculation from different varieties of barley growing on the farm of the Aberdeen substation.

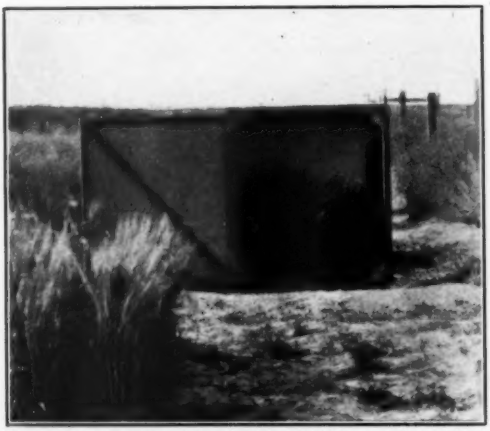

FIGURE 6.—Type of container used for subjecting inoculated wheat and barley flowers to high humidity on irrigated land

In the group subjected to a humid atmosphere after blossom inoculation, enough flowers of one of the given varieties were inoculated during one day to serve the purpose of the test. Flowers on plants growing in a clump in a row or plot on the irrigated land were inoculated. The plants of the high-humidity group were placed in chambers made of wall board and were sprayed with water twice daily for eight days after inoculation. The doors of the chambers were closed during the first three days and opened (fig. 6) during the remainder of the 8-day treatment. On the ninth day after inoculation the chambers were removed, and the plants were left to mature. Humidity and temperature records taken daily at 9 a. m., 12 m., and 5 p. m. during the 8-day period showed ranges for Federation wheat and Hanna barley of 45 to 92 per cent and 58° to 89° F., and for Moultan barley of 42 to 89 per cent and 67° to 91°. During the 3-day postinoculation period in which the doors of the chambers were closed, the relative humidity of the contained atmosphere was generally above 75 per cent.

Wheat and barley plants subjected to arid conditions were grown on the irrigated farm until about two weeks before heading. Clumps of the plants were then carefully dug and placed in galvanized-metal cans. The plants suffered no apparent injury from transplanting when the clumps were removed from the field and carefully placed in the cans so that the ball of earth incasing the roots was neither cracked nor broken. At the beginning of blossoming, the flowers were inoculated, and the plants were placed in a finely screened cage in a desert field. The cage was located about 500 yards to the windward of the irrigated plots of the experiment farm. (Fig. 7.) Test readings taken at midday on a clear typical day early in July showed a relative humidity of 13 per cent, both in the cage and at a spot in the desert 3 miles to the windward of the nearest irrigated farm. Records taken at 9 a. m., 12 m., and 5 p. m. during the 8-day period following inoculation showed humidity and temperature ranges for Federation wheat and Hanna barley of 13 to 48 per cent and 53° to 95° F., and for Moultan barley of 12 to 30 per cent and 62° to 98° F. Following inoculation the plants of this group were left in the desert until mature.

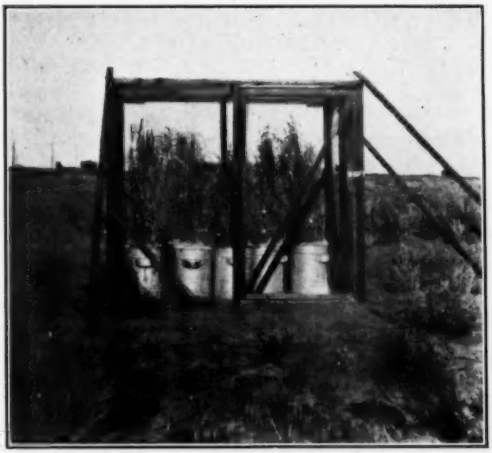

FIGURE 7.—Apparatus used in subjecting inoculated wheat and barley flowers to the low humidity of the desert

Seed of both groups was harvested at maturity and stored in sealed glass jars. The following November it was sown in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. Records on smut infection were taken after the plants were fully headed. The data are presented in Table 3.

Plants from seed developed from inoculated flowers incubated in the arid atmosphere of the desert produced much less loose smut than plants from seed developed from inoculated flowers incubated in the humid atmosphere on the irrigated land. The reduction in infection in plants of Moultan barley was especially striking (83.93 to 0 per cent). The lower humidity and higher temperature prevailing during the period in which Moultan was inoculated (Table 3) probably may be held accountable for the more effective control of smut in this variety.

In this experiment, as in the greenhouse experiment of 1927–28, there were differences in temperature as well as in humidity in the arid and humid groups. It is difficult or impossible to evaluate the influence of such differences in temperature range. Further work is necessary with apparatus that will secure variations in relative humidity with a constant temperature.

TABLE 3.—*Percentages of loose smut in Federation wheat and Hanna and Moultan barleys grown in the greenhouse from seed from flowers incubated under the given conditions of humidity and temperature for eight days after artificial inoculation at Aberdeen, Idaho, July 1, 1928*

Variety and C. I. No.	Atmospheric conditions during incubation		Plants			Heads		
	Range in relative humidity	Range in temperature	Total	Smutted		Total	Smutted	
	Per cent	*° F.*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
IN HIGH-HUMIDITY CHAMBERS ON IRRIGATED LAND								
Federation wheat (C. I. 4734)	45–92	58–89	166	116	69.88	240	164	68.33
Hanna barley (C. I. 203)	45–92	58–89	45	28	62.22	142	81	57.04
Moultan barley (C. I. 3401)	42–89	67–91	70	61	87.14	168	141	83.93
UNDER DESERT CONDITIONS								
Federation wheat (C. I. 4734)	13–48	53–95	203	37	18.23	341	49	14.37
Hanna barley (C. I. 203)	13–48	53–95	81	12	14.81	264	27	10.23
Moultan barley (C. I. 3401)	12–30	62–98	108	0	0	257	0	0

DISCUSSION

The data show that low relative humidity during the period of blossoming plays an important rôle in preventing the infection of wheat and barley with their respective loose smuts. By growing a crop in a dry area for one year it would seem possible to rid it of loose smut without seed treatment. This might have a practical use worthy of some consideration in view of the fact that the heavy annual loss from loose smut in wheat and barley goes on unchecked largely because the seed treatment recommended for control is difficult to apply and is likely to reduce germination and yield. Clean seed might be produced in arid areas either under dry farming or by proper manipulation of irrigation. The important consideration is low humidity during blossoming.

The knowledge of the effect of low atmospheric humidity on the infection of wheat by *Ustilago tritici* would seem to be particularly applicable in the case of highly susceptible varieties grown under irrigation, such as Dicklow and Federation. Dicklow is extensively grown under irrigation in southern Idaho and to a lesser extent in Montana, Utah, and Wyoming (*4*). Federation is now widely grown under irrigation in Idaho. A modification of irrigation practice to induce a low humidity during blossoming should reduce infection. The details should be determined by experiment.

SUMMARY AND CONCLUSIONS

Loose smut in wheat and barley rarely occurs in the Pacific Coast States and in other sections of the country where the relative humidity of the air is low at the flowering time of these cereals. It is at this time that the loose-smut spores are blown from diseased heads into the open blossoms, where normally they germinate and produce infection hyphae that enter the developing kernel.

When flowers of the three club wheats Hybrid 128, Jenkin, and Little Club were inoculated under humid conditions in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va., the resulting seed produced from 91 to 98 per cent smutted plants. These wheats are widely grown in one or more of the Pacific Coast States and rarely produce loose smut there.

Flowers of Little Club wheat were exposed for eight days after inoculation to low (11 to 30 per cent) and high (56 to 85 per cent) ranges in relative humidity. Seed from flowers subjected to the low humidity produced 21.90 per cent smutted plants, while seed from flowers subjected to the high humidity produced 93.96 per cent smutted plants. In this experiment the low humidity was obtained by recirculating air over calcium chloride in a closed chamber.

Daily microscopic examinations of inoculated flowers showed that in plants exposed to humid conditions many spores were in the early stages of germination one day after inoculation, and that on the third day following inoculation approximately 90 per cent of the spores had germinated and produced long germ tubes. In the group exposed to arid conditions, spores in the early stages of germination were not found until the third day after inoculation, and only a few with long germ tubes were found seven days after inoculation. These observations seem to indicate that in the presence of appropriately low relative humidity loose smut in wheat is held in check because the smut spores either fail to germinate or germinate too slowly to enable infecting hyphae to reach the ovary during the short period in which it is vulnerable.

At Aberdeen, Idaho, flowers of Federation wheat and of Moultan and Hanna barleys were inoculated on plants on the desert and on plants on irrigated land. In each variety the seed from flowers inoculated and incubated under desert conditions produced a much lower percentage of smutted plants and heads than the seed from flowers inoculated and incubated under irrigation conditions. Plants from seed from the inoculated flowers of Moultan barley incubated under high humidity produced 83.93 per cent of smutted plants as compared with 0 per cent of smutted plants from seed from inoculated flowers incubated at a low humidity.

In the experiments involving a comparison of the effects on infection of high or low ranges of humidity, the latter were accompanied by ranges of temperature greater than those accompanying the high humidity ranges. However, the ranges of temperature did not extend beyond the limits for germination of the spores of *Ustilago tritici* and *U. nuda* except in the case of Moultan barley. The possible influence of these temperature differences must be recognized, but they can not be evaluated in these experiments.

The data suggest a practical method for producing seed stocks free from loose smut by growing them under conditions of low relative humidity during the blossoming period.

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EFFECTS OF COD-LIVER OIL AND ULTRA-VIOLET IRRADIATION, AS INFLUENCED BY OYSTER SHELL, IN THE DIET OF CONFINED LAYING HENS[1]

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INTRODUCTION

The purpose of the study herein reported was to determine the comparative values of cod-liver oil and ultra-violet irradiation in supplying the nutritional factors of which laying hens are deprived when they are confined without access to direct sunlight or green feed. During the course of the investigation it was observed that the presence of oyster shell in the diet had such an effect that it appeared desirable to include a study of this factor in the experimental work.

The results of a large number of investigations, relating to the comparative values of cod-liver oil and ultra-violet irradiation, have been published since the writers began their study of the problem. However, in these investigations the presence of certain mineral supplements in the diet as a possible factor affecting the results of feeding cod-liver oil or administering ultra-violet irradiation does not appear to have received much attention. Therefore the writers made a rather detailed analysis of the effect of the presence of oyster shell in the diet in addition to comparing the effects of the cod-liver oil and the ultra-violet irradiation.

REVIEW OF PREVIOUS INVESTIGATIONS

The importance of calcium in the diet of the laying hen has long been recognized. As early as 1892 a bulletin of the New York State Agricultural Experiment Station (*11*)[2] reported the results of a feeding experiment in which a grit consisting of ground oyster shells was compared with one consisting of ground glass. The hens receiving oyster shell produced more eggs, and the eggs had better shells. In 1919, Wheeler (*31*) found that a deficiency of inorganic calcium depresses egg production in hens and ducks. The results of extensive investigations relating to the calcium metabolism of laying hens reported by Buckner, Martin, and associates at the Kentucky station (*2, 3, 4, 5, 6, 7, 8, 9*) show that the feeding of calcium carbonate results in increased egg production, larger eggs, heavier shells, increased hatchability of the eggs, and greater size and vigor of the chicks hatched. Halpin (*15*) found that feeding oyster shell increases the thickness of the egg shell. Robertson and Baskett (*27*) have recently reported that feeding minerals increases egg production and egg size and improves the general health of the birds.

As vitamin D is very important in regulating the calcium metabolism of the growing chick, a plentiful supply of this vitamin is as

[1] Received for publication Mar. 9, 1931; issued October, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 533.

essential to the laying hen as an adequate supply of calcium. The results of a large number of experiments published in recent years, although not always in exact agreement, show definitely that a lack of vitamin D in the diet, or its equivalent in ultra-violet irradiation, impairs the health of the laying hen and depresses the physiological processes involved in egg production. The New Jersey station (*30*) has reported that the exclusion of the antirachitic factor from the diet results in decreased egg production and hatchability. Taylor and Martin (*29*) recently found that a lack of vitamin D or an insufficient supply of available calcium will result in the formation of thinner egg shells. Wheeler (*32*) recently found that excluding direct sunlight from laying hens impairs egg production and hatchability.

In 1924 the Kansas station (*13*) reported that the artificial irradiation of birds not having access to direct sunlight increases egg production and the hatchability of the eggs. Hughes and Payne (*18*) found that irradiation increases egg production, shell weight, and hatchability. Hughes, Payne, and Latshaw (*19*) compared artificial irradiation with exposure to direct sunlight as a source of vitamin D. The birds exposed to direct sunlight produced more eggs, but the hatchability was lower. Hart, Steenbock, Lepkovsky, Kletzien, Halpin, and Johnson (*17*) found that irradiation increases egg production, shell weight, and hatchability. Only a slight increase in fertility was noted.

In a more recent publication Hart, Scott, Halpin, and Johnson (*16*) reported that egg production and shell weight are increased by feeding cod-liver oil or by administering ultra-violet irradiation. The Ohio station (*33*) reported that egg production is increased by feeding cod-liver oil or irradiating the birds. It was found that irradiating the birds increases the hatchability of the eggs but that feeding cod-liver oil has no beneficial effect on hatchability.

Kaupp (*21*) found that feeding cod-liver oil increases the fertility and hatchability of the eggs. Feeding a mineral supplement together with cod-liver oil was found to increase egg production and improve the general condition of the birds. The Idaho station (*20*) reported that feeding cod-liver oil increases egg production if the birds are not exposed to direct sunlight. The South Carolina station (*24*) reported that feeding cod-liver oil to the laying stock during the winter months increases egg production, fertility, and hatchability.

Much of the beneficial action of cod-liver oil is doubtless due to the presence of other factors, vitamin A in particular, in addition to vitamin D. Stuart (*28*) found that feeding cod-liver oil increased egg production and hatchability when the birds were exposed to direct sunlight but were deprived of green feed. Bobby (*1*) reported that feeding cod-liver oil to confined pullets brought up their egg production to that of birds which were permitted to go out on grass runs. Whether or not the confined birds received any direct sunlight was not stated, however.

In some of the other experimental work summarized the birds probably had access to direct sunlight, but the conditions under which the birds were kept were not always described in detail.

Feeding cod-liver oil appears to have no beneficial action on birds having access to both direct sunlight and green feed. Hall (*14*) found that feeding cod-liver oil did not benefit birds receiving direct sunlight and sprouted oats. Orr, Crichton, and associates (*26*) re-

ported that feeding cod-liver oil had no favorable effect on egg production or hatchability in the case of birds having access to direct sunlight and limestone. Other details relating to the nutrition of the birds are not given.

Elford (*12*) reported that feeding cod-liver oil does not increase egg production or shell thickness. The experimental conditions were not described. Mercer and Tozer (*23*) found that neither irradiating the birds nor feeding cod-liver oil had any favorable effect when the birds were permitted to run on range. The Indiana station (*10*) reported that feeding cod-liver oil did not improve egg production, fertility, or hatchability when the birds had access to direct sunlight. Kempster (*22*) reported that feeding cod-liver oil did not markedly affect the egg production of birds kept in muslin-front houses and allowed range during the day. However, birds kept behind glass produced more eggs when cod-liver oil was included in the diet.

EXPERIMENTAL PROCEDURE

On September 14, 1927, 90 Rhode Island Red pullets, approximately 5 months of age, were taken from the chick-growing range at the United States Animal Husbandry Experiment Farm, Beltsville, Md., and distributed, 15 to a pen, among 6 pens on the second floor of a frame building where they were confined until August 12, 1929, almost two years. For the sake of brevity, the pens will hereafter be designated by their numbers, 60, 61, 62, 63, 64, and 65. Each pen measured approximately 8 by 11 feet. The windows were so arranged that all sunlight reaching the pens passed through glass. The section of the building in which the birds were kept was not heated. However, the heat from the lower story of the building, which was used as a brooder house, slightly warmed the experimental pens during cold weather.

Until October 1, 1927, the birds received their regular growing diet, except that, for the purpose of retarding egg production, meat scraps were omitted from the mash several weeks before the birds were moved from the growing range to the experimental pens. From October 1, 1927, to January 3, 1928, the birds in all six pens received, ad libitum, an all-mash diet of the following composition:

	Per cent
Ground wheat	28.0
Yellow corn meal	24.0
Rolled oats	15.0
Wheat middlings	7.5
Fish meal	5.5
Meat scraps (55 per cent protein)	5.5
Dried skim milk	4.5
Alfalfa meal	2.5
Wheat bran	1.5
Dried buttermilk	1.5
Ground limestone	1.8
Steamed bone meal	1.8
Common salt	0.9
Total	100.0

This mixture had given very satisfactory results when fed to laying hens kept under normal conditions. It contained approximately 11.02 per cent moisture, 18.44 per cent crude protein, and 6.85

per cent ash. (These percentages are averages of determinations made on samples taken at regular intervals during the course of the experiment.) In addition to this diet the birds in pens 63, 64, and 65 received crushed oyster shell ad libitum.

On January 3, 1928, the birds in pens 62 and 65 received 2 per cent, by weight, of cod-liver oil added to the mash, and those in pens 60 and 63 received 15 minutes of ultra-violet irradiation daily, except on Sundays, at a distance of 3 feet from the machine. A carbon-arc machine burning therapeutic A carbons was used to furnish the irradiation. This type of carbon is designed to furnish ultra-violet irradiation of only the same strength as that supplied by natural direct sunlight. On June 11, 1928, this machine was replaced by a quartz-mercury machine. The carbon-arc machine was returned February 9,1929, but therapeutic C carbons were substituted for the A carbons previously used. The C carbons contain iron, nickel and aluminum in the core. Silicon is also present in the carbon. These carbons are designed to give more nearly equal quantities of ultra-violet irradiation throughout the physiologically active range than any other.

TABLE 1.—*Outline of experimental procedure followed in the various pens*

Date	Pen 60	Pen 61	Pen 62
Sept. 14, 1927	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.
Oct. 1, 1927	Began feeding experimental basal diet.	Began feeding experimental basal diet.	Began feeding experimental basal diet.
Jan. 3, 1928	Began irradiating birds with carbon-arc machine, using A carbons.	.....	Added cod-liver oil to basal diet.
May 2, 1928	Began feeding oyster shell	Began feeding oyster shell	Began feeding oyster shell.
June 11, 1928	Substituted quartz-mercury machine for carbon-arc machine.	.....	.....
Feb. 9, 1929	Returned carbon-arc machine. Began using C carbons.	.....	.....
Aug. 12, 1929	Ended experiment	Ended experiment	Ended experiment.

Date	Pen 63	Pen 64	Pen 65
Sept. 14, 1927	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.	Placed 15 Rhode Island Red pullets in pen; fed usual growing diet.
Oct. 1, 1927	Began feeding experimental basal diet and oyster shell.	Began feeding experimental basal diet and oyster shell.	Began feeding experimental basal diet and oyster shell.
Jan. 3, 1928	Began irradiating birds with carbon-arc machine using A carbons.	.....	Added cod-liver oil to basal diet.
May 2, 1928	Stopped feeding oyster shell.	Stopped feeding oyster shell.	Stopped feeding oyster shell.
June 11, 1928	Substituted quartz-mercury machine for carbon-arc machine. Began feeding oyster shell again.	Began feeding oyster shell again.	Began feeding oyster shell again.
Feb. 9, 1929	Returned carbon-arc machine. Began using C carbons.	.....	.....
Aug. 12, 1929	Ended experiment	Ended experiment	Ended experiment.

It is readily apparent that the treatment given the birds in pens 63, 64, and 65 was, respectively, identical with that given the birds in pens 60, 61, and 62 except that the three first mentioned received oyster shell. During the first few months of the experiment it became evident that the birds in pens 63 and 64 were giving better results than

those in pens 60 and 61. To determine whether oyster shell was responsible, it was removed from pens 63, 64, and 65 on May 2, 1928, and placed in pens 60, 61, and 62. The effects of this change were so pronounced that on June 11, 1928, it seemed desirable to feed oyster shell to the hens in all six pens. No other changes were made in the feeding throughout the experiment.

A brief outline of the experimental procedure followed is given in Table 1.

Observations were made, and records kept, of egg production, egg weight, weight of eggshell, fertility and hatchability of eggs, and health and condition of the birds. For the sake of convenience each phase of the experiment will be considered separately.

EGG PRODUCTION

Trap-nest records were kept of all birds. The cumulative egg production was computed at 28-day intervals and reduced to a per bird basis. The cumulative egg production per bird for pens 60, 61, and 62 has been plotted in Figure 1, A.[3]

Since the ordinates of the curves represent cumulative egg production, the rate of egg production at any time is given by the slopes of the curves. It is at once apparent that the ultra-violet irradiation had very little, if any, effect upon egg production during the winter months of the first year of the experiment. The cod-liver oil, on the other hand, immediately increased the rate of egg production, as is shown by the sudden rise of the curve for pen 62. It is of particular interest to notice the increase in rate of egg production in pens 60 and 61 after the birds began receiving oyster shell. The addition of the oyster shell had no beneficial effect on the hens receiving cod-liver oil. This pen showed a slight decrease in rate of egg production immediately after oyster shell was included in the diet, but decreases in rate of egg production at this time of the year are to be expected among birds kept under normal conditions.

The rate of egg production in pen 60 does not appear to have been affected by the ultra-violet irradiation until January, 1929, or one year after the irradiation was begun. Although the total number of eggs laid up to this time was greater than that in pen 61 (basal diet alone), this difference was due to the fact that the birds in pen 60 began to lay a little earlier. From the beginning of irradiation until molting the rate of egg production was approximately the same in both pens. After January, 1929, however, the superiority in egg production of pen 60 over pen 61 was evident. It was not until the last few months of the experiment that the rate of egg production in pen 61 equaled that in pen 60.

A careful study of Figure 1, B, is very enlightening regarding the importance of oyster shell in the birds' diet. Although the beneficial effects of irradiation and cod-liver oil are discernible from the beginning, nevertheless the removal of the oyster shell from the pens caused the differences among them to be greatly accentuated. The return of the oyster shell to the pens was followed by increases in rate of egg production, which made the rate of egg production very nearly the same in all three pens. The effects of the oyster shell

[3] Lack of space prevents the publication, in tabular form, of the data used in the construction of Figures 1-6. The original data may be obtained by communicating with the authors at the Animal Husbandry Experiment Farm, Beltsville, Md.

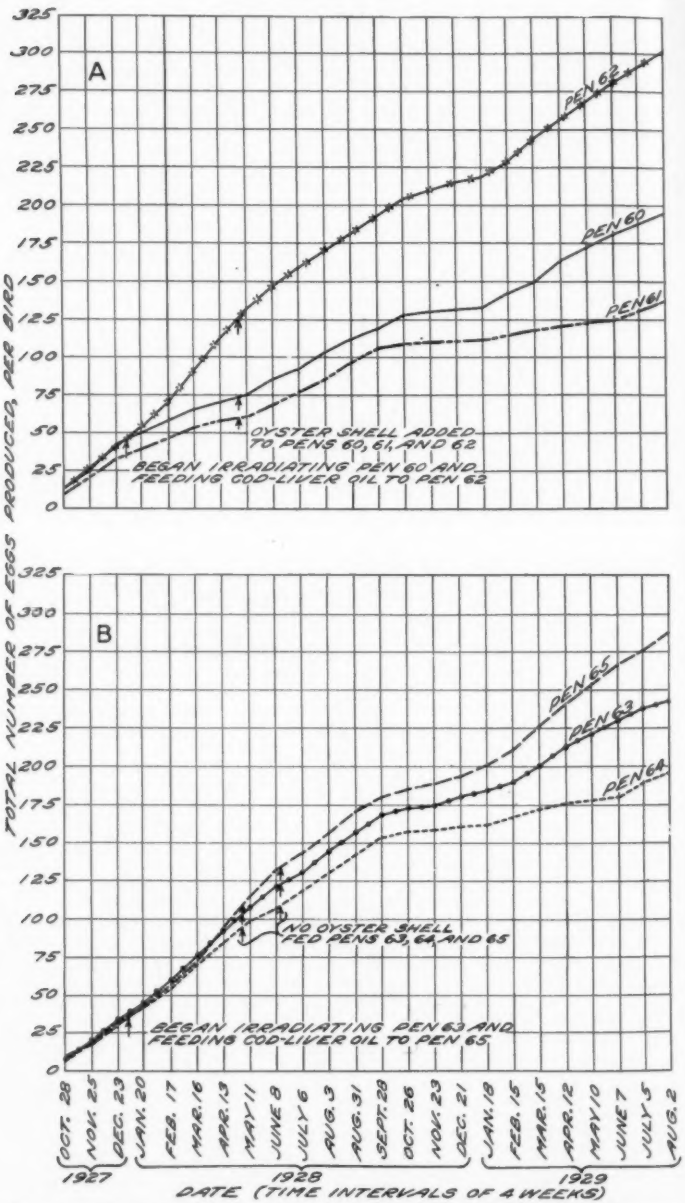
A
PEN 62
PEN 60
PEN 61
OYSTER SHELL ADDED TO PENS 60, 61, AND 62
BEGAN IRRADIATING PEN 60 AND FEEDING COD-LIVER OIL TO PEN 62
B
PEN 65
PEN 63
PEN 64
NO OYSTER SHELL FED PENS 63, 64, AND 65
BEGAN IRRADIATING PEN 63 AND FEEDING COD-LIVER OIL TO PEN 65
TOTAL NUMBER OF EGGS PRODUCED, PER BIRD
0 25 50 75 100 125 150 175 200 225 250 275 300 325
OCT. 28 NOV. 25 DEC. 23 JAN. 20 FEB. 17 MAR. 16 APR. 13 MAY 11 JUNE 8 JULY 6 AUG. 3 AUG. 31 SEPT. 28 OCT. 26 NOV. 23 DEC. 21 JAN. 19 FEB. 15 MAR. 15 APR. 12 MAY 10 JUNE 7 JULY 5 AUG. 2
1927 1928 1929
DATE (TIME INTERVALS OF 4 WEEKS)

FIGURE 1.—Cumulative egg production per bird: A, Pens in which birds received no oyster shell during the early part of the experiment—60 (irradiated), 61 (basal diet alone), and 62 (cod-liver oil added); B, pens in which birds received oyster shell from the beginning of the experiment—63 (irradiated), 64 (basal diet alone), and 65 (cod-liver oil added)

were most pronounced in pen 64 (basal diet alone) and least pronounced in pen 65 (cod-liver oil added). No significant differences among the three pens can be observed for the remainder of the first year of the experiment. During the second year of the experiment the cod-liver oil and the ultra-violet irradiation showed beneficial effects.

Figure 2 is presented to show the extent to which comparable pens were influenced by the oyster-shell feeding. Except for the two pens of birds receiving cod-liver oil, those receiving the oyster shell had higher rates of egg production during the winter months of the first year of the experiment. After oyster shell was placed in all the pens the rate of egg production became the same in the two pens compared for each group as is shown by the nearly parallel, cumulative, egg-production curves after this time. In the case of the two pens of birds receiving cod-liver oil, no beneficial effects from the oyster shell are observable. The two curves are parallel for almost the entire length of the experiment and would have coincided if the birds in both pens had begun to lay at the same time.

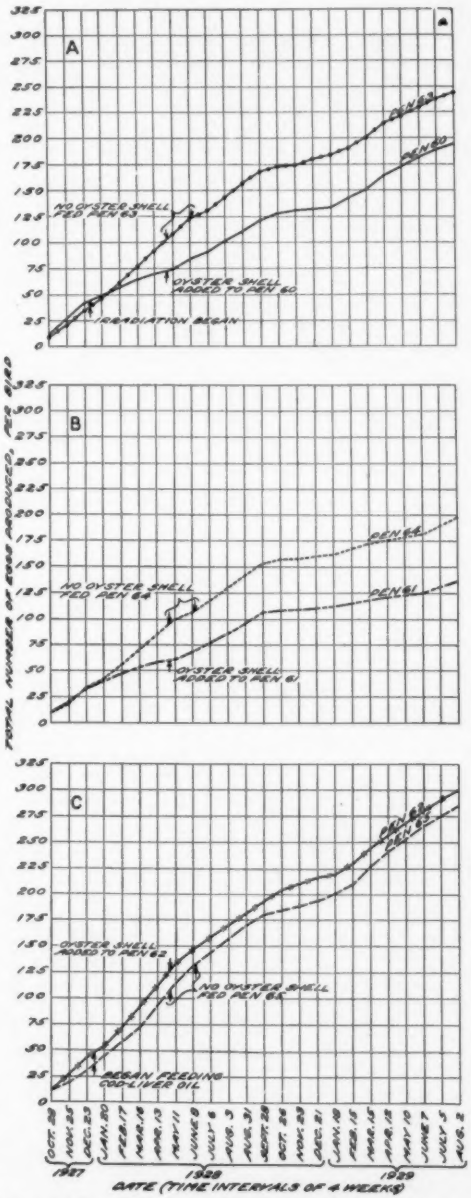

FIGURE 2.—Comparison of cumulative egg production per bird: A, Pens 60 and 36 (irradiated); B, pens 61 and 64 (basal diet alone); C, pens 62 and 65 (cod-liver oil added)

EGG WEIGHT

All eggs laid in the six pens were weighed daily. The weighings were made during the morning after the day the eggs were laid except on Sundays and holidays. On such occasions the eggs were weighed a day later. The weights were grouped by 28-day periods, and the

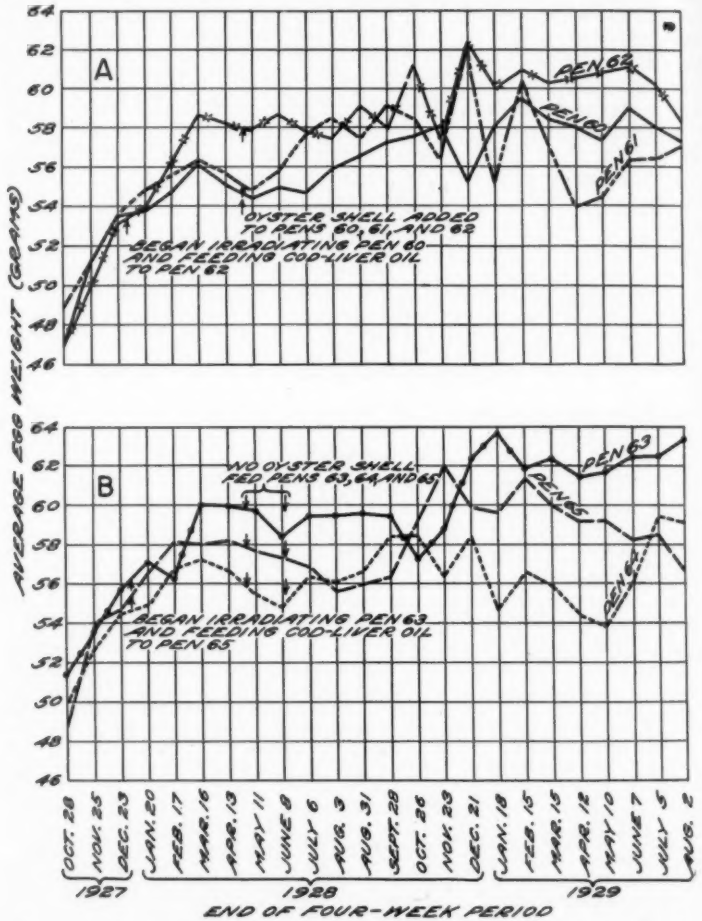

FIGURE 3.—Average egg weights: A, pens in which the birds received no oyster shell during the early part of the experiment—60 (irradiated), 61 (basal diet alone), and 62 (cod-liver oil added); B, pens in which the birds received oyster shell from the beginning of the experiment—63 (irradiated), 64 (basal diet alone), and 65 (cod-liver oil added)

average egg weight for each pen was calculated for each period. The averages are shown in Figure 3.

It is evident that the egg weights were rather variable. Figure 3, A, shows the eggs to have been consistently heavier in pen 62 (cod-liver oil added) than in pens 60 (irradiated) and 61 (basal diet alone).

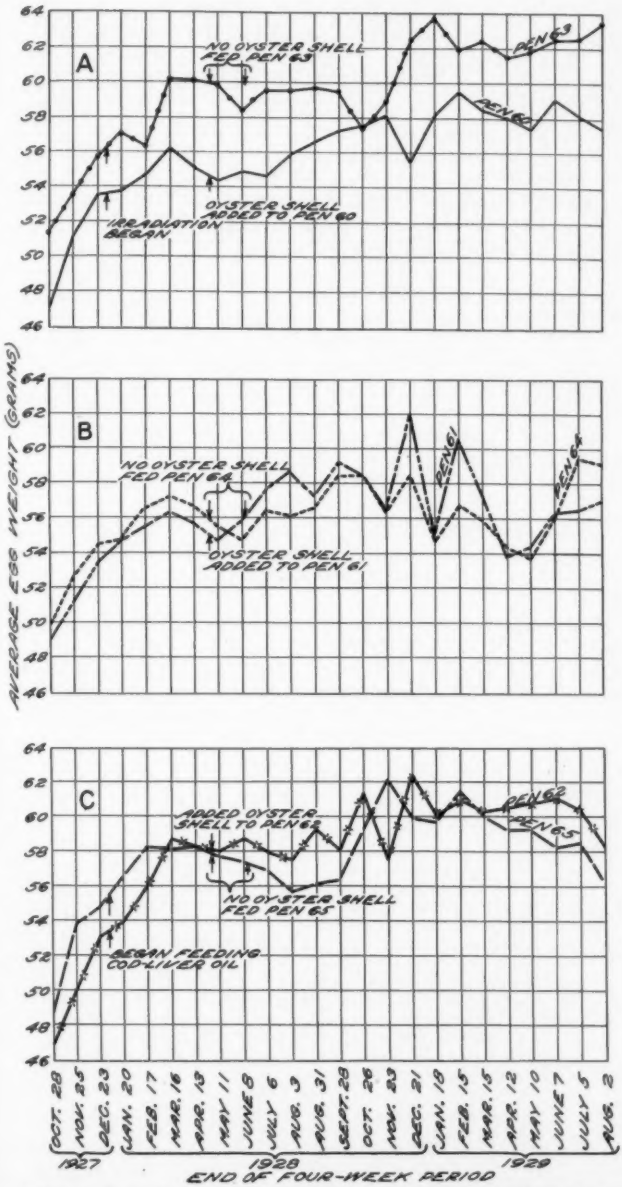

FIGURE 4.—Comparison of average egg weights: A, Pens 60 and 63 (irradiated); B, pens 61 and 64 (basal diet alone); C, pens 62 and 65 (cod-liver oil added)

The addition of the oyster shell seems to have increased the weight of the eggs in pen 61 but appears to have had no immediate effect, if any, in pen 60. The eggs from pen 61 were heavier than those from pen 60 most of the time.

These results are not in agreement with those obtained in pens 63, 64, and 65. Figure 3, B, shows the eggs from pen 63 (irradiated) to have been, in general, the heaviest and those from pen 64 (basal diet alone) the lightest. The weights of the eggs from pen 64 show a sudden increase during the last few months of the experiment. The same is true to a lesser degree of pen 61. In Figure 1 it may be observed that this increase in egg weight was correlated with the increased egg production noted in these two pens near the end of the experiment.

In Figure 4 are compared the weights of the eggs obtained from the pens of birds receiving the same treatment. Except for the two pens receiving the ultra-violet irradiation, no considerable differences in egg weight existed between the comparable pens. However, the initial egg weights in every instance were higher in those pens which received oyster shell from the very beginning of the experiment. The weights of the eggs from pen 61 (basal diet alone) showed an immediate increase after oyster shell was placed in the pen. Before the oyster shell was placed in pen 62 (cod-liver oil added) the eggs from this pen weighed as much as those from pen 65 (cod-liver oil added), but this is in agreement with the results previously noted under Egg Production. It will be recalled that when cod-liver oil was fed, the addition of oyster shell to the diet exerted no further beneficial effect.

The egg weights obtained in pen 63 (irradiated) appear too high to be due entirely to the experimental treatment which the birds in this pen received. They were probably affected by factors which were not controlled during the experiment.

THICKNESS OF EGGSHELLS

A schedule was adopted by which all eggs were saved during 14-day periods 28 days apart. There were 16 such periods, as follows (all dates are inclusive):

Period	1	Oct. 29, 1927–Nov. 11, 1927
	2	Dec. 10, 1927–Dec. 23, 1927
	3	Jan. 21, 1928–Feb. 3, 1928
	4	Mar. 3, 1928–Mar. 16, 1928
	5	Apr. 14, 1928–Apr. 27, 1928
	6	May 26, 1928–June 8, 1928
	7	July 7, 1928–July 20, 1928
	8	Aug. 18, 1928–Aug. 31, 1928
	9	Sept. 29, 1928–Oct. 12, 1928
	10	Nov. 10, 1928–Nov. 23, 1928
	11	Dec. 22, 1928–Jan. 4, 1929
	12	Feb. 2, 1929–Feb. 15, 1929
	13	Mar. 16, 1929–Mar. 29, 1929
	14	Apr. 27, 1929–May 10, 1929
	15	June 8, 1929–June 21, 1929
	16	July 20, 1929–Aug. 2, 1929

All eggs laid during these periods were broken, after being weighed, and the shells were carefully washed free from all albumen. After being permitted to dry in the air, the shells were weighed individually on a balance sensitive to 0.05 gm.

The measure of shell thickness adopted by the writers requires some explanation. It has been a rather common practice to compute, as a measure of shell thickness, the number of grams of shell per unit weight of egg. One of the primary requisites of a unit of measurement is that the number of units employed to designate a quantity be directly proportional to the quantity which is being measured. Obviously, the thickness of the shell depends upon the weight of

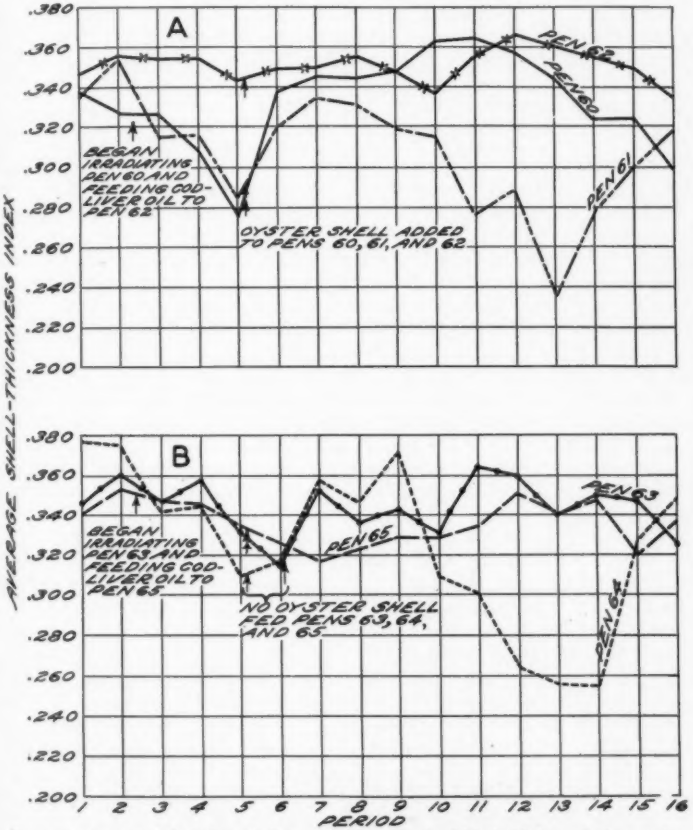

FIGURE 5.—Average shell-thickness indices of eggs: A, Pens in which the birds received no oyster shell during the early part of the experiment—60 (irradiated), 61 (basal diet alone), and 62 (cod-liver oil added); B, pens in which the birds received oyster shell from the beginning of the experiment—63 (irradiated), 64 (basal diet alone), and 65 (cod-liver oil added)

shell per unit area of egg. If the egg were a sphere, its area would, of course, be proportional to the two-thirds power of its weight. The egg is not a sphere, but Murray (*25*) has shown that the area of the surface of an egg (in square centimeters) is very nearly equal to 5.07 times the two-thirds power of the weight of the egg (in grams).

For the purpose of the present investigation it seemed more desirable to express the area of the egg as a function of the weight

of the contents of the egg rather than as a function of the weight of the entire egg. No work appears to have been reported in which the area of the egg has been expressed in this manner. However, by dividing the weight of the shell by the two-thirds power of the weight of the contents of the egg, a shell-thickness index is obtained which is proportional to the weight of shell per unit area of egg. For the purpose of comparing the shell thickness of the eggs from the various pens this shell-thickness index is quite satisfactory.

Some idea of the meaning of the index can be obtained by calculating its value for an egg having a good shell. For a 57-gm. egg having a shell weighing 5.7 gm., the index, I, was calculated as follows:

$$I = \frac{\text{weight of shell}}{(\text{weight of contents})^{\frac{2}{3}}} = \frac{5.7}{(51.3)^{\frac{2}{3}}} = \frac{5.7\sqrt[3]{51.3}}{51.3} = 0.413$$

Evidently, then, an index of about 0.400 denotes an eggshell as good as is usually produced under ordinary feeding conditions.

Figure 5, A, presents the average shell-thickness indices for the eggs from pens 60, 61, and 62 for each period. Again the effects of the oyster shell are strikingly in evidence. The eggs from pens 60 (irradiated) and 61 (basal diet alone) showed increases in shell thickness immediately after the addition of the oyster shell. The oyster shell, as before, seemed to have had no effect on the eggs from pen 62 (cod-liver oil added). The ultra-violet irradiation had no effect on the eggs from pen 60 until after the addition of the oyster shell. Between the seventh and thirteenth periods the shells from pen 61 (basal diet alone) continuously became thinner. The sudden increase in shell thickness of the eggs in pen 61 during the last few months of the experiment occurred simultaneously with the increases in egg production and egg weight to which reference has already been made.

Figure 5, B, presents the shell-thickness indices for pens 63, 64, and 65. During the first nine periods no consistent differences among these three pens can be found. This is in agreement with the results noted under egg production when oyster shell was fed from the beginning of the experiment. From the ninth to the fourteenth periods the shells from pen 64 (basal diet alone) became consistently thinner. After the fourteenth period the shells from this pen suddenly became thicker, as in pen 61, until at the end of the experiment they were thicker than those produced in any of the other five pens.

In Figure 6 are compared the average shell-thickness indices obtained from the eggs in the pens designated. Except in the case of the two pens receiving cod-liver oil, the shell thickness of the eggs was greater during the first five periods in the pens receiving oyster shell. After all the pens began receiving oyster shell there seemed to be no significant differences between the comparable pens.

FERTILITY AND HATCHABILITY OF EGGS

All eggs laid during the 10 days immediately following each shell-weighing period were used for incubation. Male birds had been placed in the pens a few weeks before the first eggs were to be saved for this purpose. The males were moved from one pen to another each day. For several periods, when very few eggs were being produced, the incubation test was omitted.

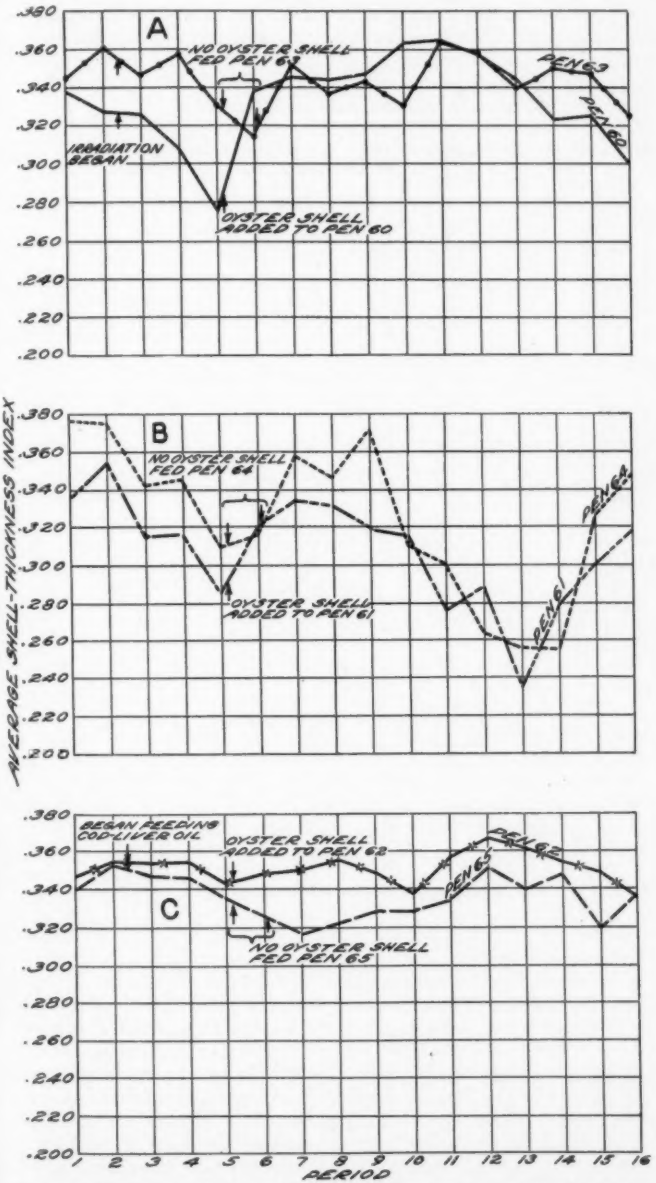

FIGURE 6.—Comparison of average shell-thickness indices of eggs: A, Pens 60 and 63 (irradiated); B, pens 61 and 64 (basal diet alone); C, pens 62 and 65 (cod-liver oil added)

Table 2 gives the averages of the results for the first and second years of the experiment. For the first year no consistent differences could be observed among pens 60, 61, and 62. Pens 63, 64, and 65 showed no consistent differences in fertility, but the percentage of good chicks hatched was higher in pens 63 (irradiated) and 65 (cod-liver oil added) than in pen 64 (basal diet alone).

TABLE 2.—*Effect of cod-liver oil feeding to and ultra-violet irradiation of hens on the fertility and hatchability of the eggs*

FIRST YEAR

Treatment and pen No.	Eggs set	Fertile eggs		Good chicks hatched		
				Total	Percentage of total eggs set	Percentage of fertile eggs
Irradiated:	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Per cent*
60	199	177	88.9	91	45.7	51.4
63	260	266	91.7	145	50.0	54.5
Basal diet alone:						
61	174	151	86.8	83	47.7	55.0
64	326	279	85.6	135	41.4	48.4
Cod-liver oil added:						
62	408	350	85.8	208	51.0	59.4
65	260	213	81.9	130	50.0	61.0

SECOND YEAR

Irradiated:						
60	155	138	89.0	96	61.9	69.6
63	209	183	87.6	100	47.8	54.6
Basal diet alone:						
61	41	29	70.7	11	26.8	37.9
64	69	57	82.6	30	43.5	52.6
Cod-liver oil added:						
62	209	158	75.6	113	54.1	71.5
65	205	156	76.1	97	47.3	62.2

For the second year of the experiment the effects of the cod-liver oil and the ultra-violet irradiation upon hatchability are apparent, but no consistent differences in fertility can be noted. However, the number of eggs produced by the birds receiving only the basal diet was very small, and those eggs were produced by only a few birds which apparently suffered least from the deficiencies of the diet.

HEALTH AND CONDITION OF THE BIRDS

All birds were weighed individually once every two weeks with a spring balance sensitive to 5 gm.

Table 3 presents the average live weights of the birds. The birds receiving cod-liver oil were consistently heavier, but the ultra-violet irradiation does not appear to have had much effect. It was not until about April, 1929, 19 months after the beginning of the experiment, that the birds on the basal diet alone were significantly lighter than those receiving the irradiation. During the early part of the experiment, as in the case of egg production and shell thickness, there was very little difference among the three pens in which the birds received oyster shell from the beginning of the experiment. However, the addition of oyster shell to pens 60, 61, and 62 does not seem to have had any effect on live weight.

TABLE 3.—*Effect of cod-liver oil feeding and ultra-violet irradiation on the average live weight and the mortality of hens*

Date of weighing	Average live weights of birds receiving no oyster shell during the early part of the experiment			Average live weights of birds receiving oyster shell from the beginning of the experiment		
	Pen 60 (irradiated)	Pen 61 (basal diet alone)	Pen 62 (cod-liver oil added)	Pen 63 (irradiated)	Pen 64 (basal diet alone)	Pen 65 (cod-liver oil added)
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Oct. 1, 1927	2,387	2,359	2,368	2,366	2,371	2,404
Oct. 15, 1927	2,511	2,412	2,487	2,466	2,432	2,501
Oct. 29, 1927	2,474	2,467	2,600	2,599	2,545	2,514
Nov. 12, 1927	2,652	2,576	2,679	[a] 2,675	2,595	2,531
Nov. 26, 1927	2,696	2,666	2,720	2,553	2,638	2,671
Dec. 10, 1927	2,612	2,621	2,693	2,670	2,592	2,679
Dec. 27, 1927	2,614	2,607	2,653	[a] 2,660	2,552	2,623
Jan. 5, 1928 [b]	2,610	2,475	2,703	2,668	2,657	[a] 2,734
Jan. 21, 1928	2,635	2,649	2,756	2,752	2,657	2,724
Feb. 4, 1928	2,656	2,760	2,864	2,814	2,798	2,849
Feb. 18, 1928	2,720	2,771	2,852	2,739	2,811	2,843
Mar. 3, 1928	2,700	2,713	2,912	2,727	2,849	2,889
Mar. 17, 1928	2,618	2,688	2,934	2,705	2,700	2,936
Mar. 31, 1928	2,529	2,626	2,982	2,748	2,697	3,021
Apr. 14, 1928	2,473	2,560	2,978	2,626	2,774	2,850
Apr. 30, 1928	2,499	2,472	2,987	2,648	[a] 2,720	2,935
May 12, 1928 [c]	2,627	2,631	2,954	2,667	2,632	[a] 2,834
May 26, 1928	2,549	2,587	2,889	[d] 2,488	2,500	2,793
June 9, 1928	2,548	2,502	[a] 2,811	[a] 2,511	2,521	2,732
June 23, 1928 [e]	2,484	2,545	2,902	2,462	2,523	2,712
July 7, 1928	2,580	2,493	2,734	2,428	[a] 2,512	2,717
July 21, 1928	2,493	2,471	2,691	2,487	2,515	2,653
Aug. 4, 1928	2,620	2,599	2,712	2,605	2,535	2,665
Aug. 18, 1928	2,649	[a] 2,548	2,799	2,594	2,608	2,672
Sept. 1, 1928	2,607	2,431	[a] 2,783	2,549	2,518	2,615
Sept. 15, 1928	2,729	2,551	2,838	2,693	2,677	[a] 2,708
Sept. 29, 1928	2,727	2,620	2,953	2,720	2,689	2,563
Oct. 13, 1928	2,772	2,668	2,973	[a] 2,647	2,756	2,618
Oct. 27, 1928	2,672	[a] 2,590	2,668	2,498	2,664	2,542
Nov. 10, 1928	2,670	2,645	2,771	2,542	2,658	[a] 2,646
Nov. 24, 1928	2,662	2,663	2,820	2,638	2,650	2,633
Dec. 8, 1928	2,677	2,719	2,874	2,668	2,708	2,680
Dec. 22, 1928	2,702	2,772	2,914	2,766	2,842	2,793
Jan. 5, 1929	2,791	2,797	2,950	2,644	2,817	2,841
Jan. 19, 1929	2,870	2,865	2,974	2,729	2,902	2,900
Feb. 2, 1919	2,786	2,849	3,010	2,754	2,942	2,920
Feb. 16, 1929	2,876	2,874	3,022	2,772	2,887	2,925
Mar. 2, 1929	[a] 2,901	2,812	3,002	2,753	2,883	2,926
Mar. 16, 1929	[a] 3,015	2,884	3,015	[a] 2,839	2,775	2,951
Mar. 30, 1929	2,879	[a] 2,723	2,988	[a] 2,858	2,787	2,955
Apr. 13, 1929	2,841	2,651	2,917	2,794	2,748	[a] 2,954
Apr. 27, 1929	2,857	2,545	3,053	2,674	2,710	2,924
May 11, 1929	2,869	2,590	2,992	2,880	2,665	3,008
May 25, 1929	2,869	2,622	3,033	2,916	2,755	3,049
June 8, 1929	2,780	2,622	3,003	2,859	2,740	3,020
June 22, 1929	2,742	2,605	3,034	2,861	2,695	2,989
July 6, 1929	2,793	[a] 2,661	3,008	2,868	2,738	2,896
July 20, 1929	2,680	2,608	2,883	2,804	2,664	[a] 2,906
Aug. 3, 1929	2,670	2,537	2,877	2,705	2,663	[a] 2,973
Aug. 12, 1929	[a] 2,777	2,602	2,980	2,789	2,776	3,003

[a] One bird died during the 2-week period preceding the date of the weighing.
[b] Began irradiating birds in pens 60 and 63 and feeding cod-liver oil to birds in pens 62 and 65 on Jan. 3, 1928.
[c] Stopped feeding oyster shells to birds in pens 63, 64, and 65 and began feeding them to birds in pens 60, 61, and 62 on May 2, 1928.
[d] 2 birds died during the 2-week period preceding the date of weighing.
[e] Began feeding oyster shells to birds in pens 63, 64, 65 on June 11, 1928.

Although the physical condition of the birds receiving cod-liver oil and ultra-violet irradiation was better, as indicated by their higher live weights, the mortality in these pens was not any lower. The degree of mortality in each pen is indicated in Table 3.

Beginning April 12, 1928, all birds which died were sent to the Pathological Division of the Bureau of Animal Industry for autopsy. The causes of death were varied and did not appear to be related in any way to the experimental procedure followed.

Beginning September 29, 1928, the progress of the molt was observed once every two weeks. At the time the observations were begun the birds receiving the ultra-violet irradiation and cod-liver oil were well advanced in the molt. They had continued laying for a longer time than the birds receiving the basal diet alone and probably had begun molting later. They also recovered from the effects of the molt and came back into egg production earlier.

DISCUSSION

In general, the results obtained in the study herein reported are in agreement with those obtained by other investigators under comparable conditions. The cod-liver oil appears to have been distinctly superior to the kind of ultra-violet irradiation furnished 15 minutes daily as a supplement to the basal diet used in this experiment. This does not necessarily imply that irradiation was a poorer method of supplying vitamin D, for the basal diet may have been deficient in other accessory food factors which were supplied by the cod-liver oil but not by irradiation. The work of Stuart (*28*) tends to substantiate this view.

The most marked effects of cod-liver oil and irradiation were found in egg production. Both vitamin supplements showed a very definite tendency to increase egg production.

Although irradiation and cod-liver oil appeared to have some effect in increasing egg weight, the high variability of the egg weights makes the results of doubtful significance.

Both cod-liver oil and irradiation appeared to have some effect in the formation of eggshells.

The writers were unable to demonstrate any very marked beneficial effects on fertility of the eggs by feeding cod-liver oil or by administering the kind of ultra-violet irradiation used. The hatchability of the eggs appeared to have been increased both by feeding cod-liver oil and by irradiating the birds. The small number of eggs produced by the birds receiving the basal diet detracts considerably from the significance of the results. Some individual birds apparently can withstand the effects of a deficient diet much better than others, and those eggs which were produced in the two pens where the birds received the basal diet were probably laid by the few birds which were least representative of the pens.

The cod-liver oil showed a marked influence on live weight of the birds, but irradiation does not appear to have had much effect.

The results obtained by feeding oyster shell are worthy of some attention. According to the writers' data the results can not be attributed to any deficiency of calcium in the basal diet, for, it will be recalled, oyster shell had no further beneficial effect when cod-liver oil was included in the diet. If it is assumed that the basal diet was deficient in inorganic calcium when it was fed to birds without sunlight or additional vitamins, it is necessary to account for the fact that the addition of oyster shell to the diet of the birds receiving cod-liver oil did not exert any favorable influence. If the basal diet was not deficient in inorganic calcium, there are but two alternatives to be considered.

If the cod-liver oil was more efficient than the ultra-violet irradiation, in supplying the birds with vitamin D, it is conceivable that the

birds receiving the cod-liver oil could utilize the mineral already present in the mash, whereas the other birds had to depend upon the oyster shell to a great extent. This presupposes that the mineral in the oyster shell was more available to the birds because of its physical state. If this is not the correct explanation, the only other hypothesis the writers can offer is that the oyster shell contained a small quantity of some factor which was also present in the cod-liver oil.

It is no particularly novel experience to find that oyster shell possesses nutritive properties lacking in some other common inorganic mineral supplements. Halpin (*15*) called attention to the superiority of oyster shell as a mineral supplement for the laying hen and mentioned the results of some experimental work which indicated that this superiority was not due to the iodine content of oyster shell. Whether oyster shell is merely a source of easily available calcium or whether it actually contains some factor in common with cod-liver oil can be determined only by further work on this subject.

The most surprising development during the experiment was the sudden improvement in egg production, egg weight, and shell thickness during the last few months of the experiment in the two pens where the birds received the basal diet. The writers can offer no satisfactory explanation for this. No changes had been made either in feeding or management at this time.

SUMMARY AND CONCLUSIONS

Feeding cod-liver oil or administering ultra-violet irradiation to laying hens confined without access to direct sunlight or green feed increased egg production and the thickness of the egg shells and improved the general condition of the birds. Both of these vitamin supplements also show indications of a tendency to increase egg weight and improve the hatchability of the eggs.

Cod-liver oil was superior to 15 minutes' daily administration of the type of ultra-violet irradiation used in this experiment as a supplement to the basal diet.

When no cod-liver oil was included in the diet of hens confined without access to direct sunlight, the addition of oyster shell increased egg production and thickness of the egg shell, even though a mineral supplement was present in the basal diet.

Oyster shell either is a source of easily available mineral or contains a small amount of some factor which is present in cod-liver oil.

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THINNING EXPERIMENTS IN YOUNG DOUGLAS FIR[1]

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INTRODUCTION

In the fall of 1919 and the early spring of 1920 a thinning experiment was initiated in a 9-year-old stand of Douglas fir (*Pseudotsuga taxifolia*) near Martha Creek at Wind River Forest Experiment Station,[2] Stabler, Wash. A set of three plots was laid out under the supervision of J. V. Hofmann, then in charge of the station. At the same time a set of two plots was established at the lower edge of a steep slope a short distance from the first set. During the summer of 1927 a forest fire swept over extensive areas in this neighborhood, completely destroying more than one-half of the thinned plot on the second set, together with a part of its check plot. This damage and other factors, such as the regrowth of trees not cut off closely enough to the ground and the presence of seed trees which might continue to throw seed on the area, destroyed much of the value of these plots for experimental purposes. The following discussion will deal only with the first-mentioned group of three plots, called the "piedmont plots."

On these three plots the second measurements were taken in the fall of 1924 and the spring of 1925 under the direction of L. A. Isaac and A. G. Simson, and the third measurements were taken in September, 1929, under the direction of the writer.

PURPOSE AND SCOPE OF THE STUDY

This study was started to determine the effects of thinning upon dense seedling and sapling stands of Douglas fir and to find the best methods of carrying out the thinning. On one plot (plot 1) the trees were thinned to as near an 8 by 8 foot spacing as was possible. As position rather than the characteristics of the seedlings was the chief consideration, there were in the resulting stand dominant, intermediate, and suppressed trees. The second plot (plot 3) was thinned to an approximate 8 by 8 foot spacing, but only the dominant trees were left. This plot should therefore have had an initial advantage over plot 1, in that the trees had larger average diameters and heights as well as greater vitality. The third plot (plot 2) was established as a check plot.

STAND DESCRIPTION

The stand on the experimental plots originated in 1910 after a spring slash burn. As late as 1920 seedlings were apparently still germinating, but the stand had reached a stage that made a large reduction in numbers during the next few years inevitable. It can be assumed that the stand was fairly well established in the spring of 1911, making its age for the 1919, 1924, and 1929 measurements 9, 14, and 19 years, respectively.

[1] Received for publication Mar. 17, 1931; issued October, 1931.

[2] This station is now a branch of the Pacific Northwest Forest Experiment Station.

The soil can be characterized as a gravelly sandy loam. Here and there are spots of broken rock, but no ledgy outcrops are to be seen. A ridge 5 to 10 feet high runs through the check plot. Even on so small an area there are undoubtedly small differences in site which will be reflected in the growth of the young stands. The effect, however, may not last long, as the stand may gradually build up the soil and tend to iron out the small differences which affect initial growth.

In 1919 the vegetation included vine maple (*Acer circinatum*), alder (*Alnus oregona*), elderberry (*Sambucus* sp.), and a heavy growth of fern, raspberry, lupine, and Oregon holly grape (*Berberis oregona*). In 1929 the following description of the vegetation was given:

> Underbrush, usually vine maple and willow, heavy in spots, especially in the dominant thinned stand. On plot 1 some alder and oceanspray occur in scattered clumps. Scattered red oak saplings survive from an acorn planting made in 1915; some are 10 to 15 feet tall but very slim, and others are only 2 to 3 feet tall. The ground cover consists chiefly of the raspberries, Oregon grape, rose, and bracken, with some salal, starflower, violet, grass, star-flowered Solomon's seal, everlasting, fireweed, and lupine.

No exact site determination has been made, but from comparison with standing timber in similar situations in the same valley, this must be Site II.

It was intended that the plots be 0.75 acre in size. A recheck of the boundaries showed slight discrepancies, and the following areas are taken as the corrected values: Plot 1, 0.79 acre; plot 2, 0.76 acre; and plot 3, 0.75 acre.

ESTABLISHMENT OF EXPERIMENT AND SUBSEQUENT MEASUREMENTS

1919-20 OPERATIONS

The stand on plot 1 was marked for thinning, and tallies were made for the remaining stand and for the cut trees separately. The trees were originally divided into four dominance classes, mainly on the basis of height. Originally dominant, intermediate, and suppressed classes, and an unnamed class, which included the trees just being established, were recognized. The fourth class does not appear in the subsequent remeasurements. Uncut trees were all tagged, and the diameter 1 foot above the ground and the height of each tree were taken. Of the cut trees 684 were measured in the same way.

Plot 2 was the check plot and was left untouched. The diameter 1 foot above the ground and the height of each tree were measured, but the trees were left untagged.

Plot 3 was marked so that only dominant trees were left, at an approximate 8 by 8 foot spacing. These trees were tagged, and the total height and diameter 1 foot above the ground were measured. A complete tally was made of all the cut saplings.

1924-25 OPERATIONS

On plot 1 the diameter 1 foot above the ground and the total height of each tree were measured.

One-third of the area of plot 2, or one-quarter acre, was marked off adjacent to plot 3. On this quarter acre each tree was measured for diameter and for height.

Each tagged tree on plot 3 was measured for total height and for diameter at a height of 1 foot.

1929 OPERATIONS

The trees on plot 1 were retagged, the new tag being placed at breast height. The diameter was measured 1 foot above the ground, to tie in with the former measurements, and also at breast height, at which point all future measurements would be taken. The height of every fifth tree was taken.

The trees on plot 2 were tagged at breast height to a lower diameter limit of 1.6 inches on 0.488 acre and of 2.6 inches on the remainder of the plot. The height of approximately every tenth tree was taken.

The trees on plot 3 were retagged at breast height, and the diameter 1 foot above the ground and at breast height was measured. The height of every fifth tree was taken.

On all plots the boundaries were resurveyed. An isolation strip 10 to 15 feet wide was made around plots 1 and 2. As the trees increase in size, this strip can gradually be widened.

Table 1 shows conditions on the three plots before and after thinning. The original stand conditions, as shown by the table, reveal much as to the exact comparability of the values. Of the three plots, plot 1 had the largest number of trees, but these were of the smallest average diameter and height. Plot 3 has less than half as many trees, but these were of considerably larger average diameter and greater height. The check plot was intermediate. On plot 1, thinning made little change except in the average height and diameter of all trees. These values more nearly approached those of the check plot. The average dimensions of the trees of plot 3 were noticeably greater. Intrinsic differences existed, therefore, at the establishment of the plots and are likely to be reflected in the measurements of later periods. There must be some definite reason why adjoining acres of the same stand should show such decided differences at so early an age.

TABLE 1.—*Measurements on Douglas fir thinning plots, before and after thinning, 1919–30*

[Values for actual area]

BEFORE THINNING

Plot No.	Area	Number of trees				Average diameter of trees 1 foot [a] above ground				Height of trees				Total basal area 1 foot above ground
		Dominant	Intermediate	Suppressed	All	Dominant	Intermediate	Suppressed	All	Dominant	Intermediate	Suppressed	All	
	Acre					*Inches*	*Inches*	*Inches*	*Inches*	*Feet*	*Feet*	*Feet*	*Feet*	*Sq. ft.*
1	0.79	2,073	1,794	978	4,845	1.07	0.43	0.26	0.52	6.3	3.1	1.5	4.5	7.2
2	.76	1,046	1,263	1,118	3,427	1.28	.97	.63	1.03	7.9	6.5	4.7	6.0	19.9
3	.75	1,022	558	447	2,027	1.43	1.01	.64	1.27	7.9	6.3	4.3	6.6	17.8

AFTER THINNING

1	0.79	333	148	20	501	1.08	0.40	0.25	1.04	6.4	3.8	1.6	6.3	3.0
2	.76	1,046	1,263	1,118	3,427	1.28	.97	.63	1.03	7.9	6.5	4.7	6.0	19.9
3	.75	510	.....	.....	510	1.55	.....	.....	1.74	8.0	.....	.....	8.5	8.0

[a] The basal areas were computed from the actual tree tally. This amount divided by the total number of trees gives the average basal area for the whole plot. However, the average diameters for the individual dominance classes were taken from an old computation in which the actual diameters in inches were tallied; hence, they are a tenth or two lower than they would be if computed on a basal-area basis. The average heights for the dominance classes are also based on the average for the whole group. If based upon height curves and the size of the average tree by basal area, the values would be somewhat higher.

CUMULATIVE PLOT VALUES

For comparison, the values should be translated to full-acre basis and about as many trees should be selected on the check plot as remain on the thinned area. The 500 largest trees on plot 2 at each measurement were therefore chosen. Though this manner of choice favors plot 2, since the best trees at the beginning of the experiment were not the best in the following periods, it was used because the trees on this plot were left unnumbered, and the initial 500 could not be followed. The result is that the best 500 at each measurement will have higher average diameter and height than the initial 500 would have shown.

Table 2 summarizes the results of thinning. It may be computed from this table that the basal area of plot 1, which at the beginning was only 14.9 per cent of the check plot, had in 1929 increased to 30.5 per cent. On plot 3 the increase was from 41.4 per cent to 58.5 per cent. Hence trees on both plots are growing somewhat faster than those in the unthinned stand, even though this is not so obvious from the average dimensions. In average height trees on plot 1 approximate the average for the whole unthinned stand, whereas those on plot 3 compare more closely with the 500 best trees in both the 1924 and 1929 measurements. The diameter of trees on plot 1 is increasing more slowly than that of the 500 best trees on the check plot. On plot 3 the increase is substantially greater, especially in view of the fact that the value of the 500 best trees on the check plot is larger than it would be if the trees originally measured had been used in each subsequent measuring. Hence, as is shown by Table 2, thinning can substantially increase the diameter growth, even of young stands, especially if the right trees are left. The spacing is much less important than the character of the trees. In fact, there seems to be no virtue at all in thinning unless good trees are left. No determinable effect of thinning upon height growth is shown, since plot 1 follows one series of values and plot 3 the other.

TABLE 2.—*Measurements on Douglas fir thinning plots, at 5-year intervals*

[Values on acre basis]

Year	Plot No.	Age	Trees	Average diameter				Average height		Total basal area		Cubic volume above 2 inches diameter breast high
				All trees 1 foot above ground	All trees at breast height	500 best at 1 foot above ground	500 best at breast height	All trees	500 best trees	1 foot above ground	At breast height	
		Years	*Number*	*Inches*	*Inches*	*Inches*	*Inches*	*Feet*	*Feet*	*Sq. ft.*	*Sq. ft.*	*Cu. ft.*
1919	1	9	635	1.06	.....	.....	.....	6.3	.....	3.9	.....	.....
1924	1	14	634	2.15	.....	.....	.....	9.8	.....	16.0	.....	.....
1929	1	19	646	3.39	2.89	.....	.....	19.8	.....	40.6	28.9	316
1920	2	9	4,503	1.03	.....	1.58	.....	6.0	9.3	26.1	.....	.....
1925	2	14	5,832	1.46	.....	2.61	.....	9.8	15.2	67.9	.....	.....
1929	2	19	4,196	.....	2.03	.....	3.53	20.0	27.8	.....	94.7	942
1920	3	9	678	1.71	.....	.....	.....	8.5	.....	10.8	.....	.....
1924	3	14	672	3.07	.....	.....	.....	15.2	.....	34.6	.....	.....
1929	3	19	677	4.42	3.88	.....	.....	27.0	.....	72.2	55.4	747

Volume above 2 inches diameter breast high on plot 1 is 33.5 per cent of that on the check and on plot 3 is 79.3 per cent. Thinning to

favor dominants has therefore given a volume more than twice that of indiscriminate thinnings. It even appears probable that the volume in the dominant stand (plot 3) may equal if not surpass that of the unthinned plot within a short time.

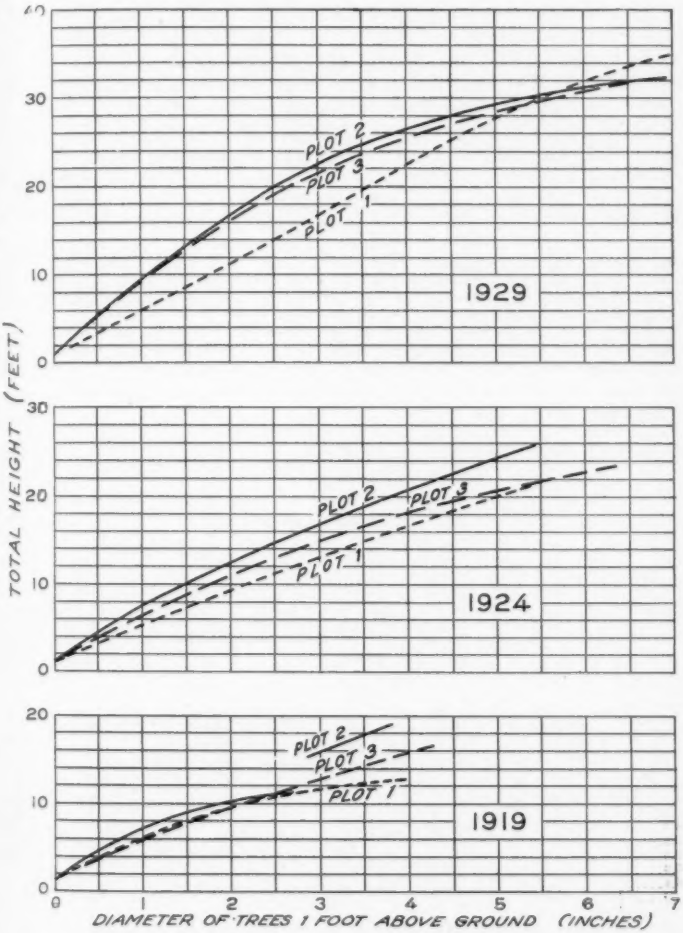

FIGURE 1.—Height curves of thinning plots in even-aged Douglas fir

HEIGHT RELATIONS

The height curves (fig. 1) for the three plots demonstrate an entirely different angle of the height relations. In 1919 differences in height were slight. Plot 2 had an advantage of 1 to 1½ feet in the central size classes. The eccentricities of the largest diameter classes are not so important, since these points are based upon a very few trees. In 1925 the check plot had apparently gained in its ad-

vantage, the plot thinned to an exact 8 by 8 foot spacing stood the lowest, and the dominant plot maintained an intermediate position. In 1929 relative positions were the same, but height in the dominant stand approached that on the check plot, and plot 1 was badly behind except in the large diameter classes. Figure 1 shows strictly a diameter-for-diameter relation and does not involve the average heights mentioned previously. The thinned trees actually have a greater diameter for their height class than do the trees on the check plot. Hence the position of the height curve has been translated forward, giving the apparent effect of height reduction. This does not indicate that height growth has been injured by thinning but rather that the height-diameter relation has been reduced for the time being. The trees on the check plot are slimmer and have a fuller bole than those on the thinned plots.

TREE SIZES

The arrangement of the trees in size classes also brings out a number of illuminating points. The different original methods of tallying diameter are reduced in Table 3 to one scheme. Because of the large number of trees in the small-size classes, a disturbance of the class range may cause a somewhat faulty redistribution in the new class ranges, but the picture presented may be considered to be accurate enough.

TABLE 3.—*Number of trees per acre by diameter classes in Douglas fir thinning plots, Columbia National Forest*

Diameter classes (inches)	Number of trees per acre in—									
	Plot 1				Plot 2			Plot 3			
	1 foot above ground			Breast high, 1929	1 foot above ground		Breast high, 1929	1 foot above ground			Breast high, 1929
	1919	1924	1929		1920	1925		1920	1924	1929	
0.0–0.4	88	6	1	15	305	793	401				
0.5–0.9	255	34	8	33	1,811	1,488	657	7			3
1.0–1.4	229	119	28	59	1,815	1,456	794	246	5		4
1.5–1.9	51	156	62	85	445	1,052	770	269	45	5	16
2.0–2.4	9	153	72	89	116	620	558	132	145	15	29
2.5–2.9	2	102	104	107	12	300	439	20	176	44	93
3.0–3.4		42	111	111	4	88	306	4	147	95	135
3.5–3.9		11	106	62		32	170		73	127	136
4.0–4.4		6	64	40			53		41	127	117
4.5–4.9		2	43	27			37		27	100	57
5.0–5.4		2	24	2			8		8	57	47
5.5–5.9			13	4			1		3	43	20
6.0–6.4			1	5			2		1	36	16
6.5–6.9			1	1						8	3
7.0–7.4			4							16	1
7.5–7.9			1							4	
Total	634	633	643	640	4,508	5,832	4,193	678	671	677	677
4 inches and above	0	10	151	79	0	0	101	0	80	391	261

The inconsistency in the total number of trees on plot 2 is partly due to the fact that only one-third of the plot was measured in 1925 and used as a sample for the whole plot, and partly to the presence of seedlings which apparently have become established since the first measurement.

Plot 1 barely shows an improvement over the check plot in the development of large-diameter classes. The number of trees 4 inches and above in diameter breast high is even slightly less. On plot 3, however, there are more than two and one-half times as many trees above the 4-inch limit as on the check plot.

A more complicated way of showing the spread of tree sizes in the stand, to show the change in their distribution with advance in age, and to elaborate upon the immediate effect of the thinning on the character of the stand, is to subject the distribution given in Table 3 to certain statistical methods which were explained in a previous publication on permanent sample plots in Douglas fir.[3] By using this method values were computed for the distributions in Table 3 and are shown in Table 4.

TABLE 4.—*Diameter distribution characteristics in Douglas fir thinning plots*

Plot and year	Standard deviation	Coefficient of asymmetry	Coefficient of excess
	Inches		
Plot 1: [a]			
1919	0.45	−0.070	+0.018
1924	.77	−.090	+.035
1929	1.29	−.062	+.021
Plot 2:			
1925	.45	−.097	+.038
1925	.75	−.099	−.002
1929	1.05	−.100	−.003
Plot 3: [a]			
1919	.43	−.103	+.054
1924	.82	−.119	+.022
1929	1.14	−.090	+.006

[a] After thinning.

Table 4 indicates the bunching of the trees in the smaller diameter classes in all three plots for all three measurements. In plot 1 the distribution apparently was made slightly more normal, since both the coefficient of asymmetry and of excess are nearer to 0 than in the check plot. But in plot 3 the opposite is true, because both coefficients are, on the whole, larger. For this young age and short period the plots show very little trend in the value of the coefficient of asymmetry, which should approach lesser negative values with increasing age. The coefficient of excess, however, tends from large positive values to smaller positive values and even to negative values. The standard deviations are only slightly affected by the thinning and retain their comparability throughout the 10 years, although plot 2 seems to be losing out.

THE AVERAGE TREE

In a stand as young as the one under study the progression throughout life of the average tree and its relation to the whole stand are interesting phenomena. On both plots 1 and 3 these relations can be further investigated, since at each measurement the trees were measured individually by number. On plot 2 further investigation will not be possible, since the trees were measured only collectively up to the last measurement.

[3] MEYER, W. H. RATES OF GROWTH OF IMMATURE DOUGLAS FIR AS SHOWN BY PERIODIC REMEASUREMENTS ON PERMANENT SAMPLE PLOTS. Jour. Agr. Research 36: 193–215, illus. 1928.

In 1920 the trees on plot 1 after thinning had an average diameter of 1.04 inches 1 foot above the ground and those on plot 3 of 1.74 inches. The difference must be assumed to result simply from the choice of the trees and not from tree development or plot conditions. On each plot 35 trees approximating this average diameter were selected; for instance, for plot 1, 35 trees of the 1-inch-diameter class in 1920 were taken from the tallies, and for plot 3 an equal number of the 1.75-inch trees were used. These trees, when traced through the following 10 years, show the extent to which individual development varies with passage of time. At the beginning of the 10 years the total range was 0.25 inch, or an approximate standard deviation of 0.1 inch. In 1924 the average size of the 35 selected trees on plot 1 was 2.39 inches and on plot 3, 3.34 inches, with total ranges of 1.8 to 3.1 inches and 2.6 to 4.5 inches, respectively.

In partial recapitulation and in an endeavor to show the various stand characteristics in their comparable position, Table 5 is introduced.

TABLE 5.—*Comparison of the development of 35 trees of the 1920 average diameter with the development of the stand as a whole; Douglas fir thinning plots, Columbia National Forest*

Plot and year	Total stand				35 trees of 1920 average			
	Diameter at 1 inch average	range	Standard deviation	Coefficient of variation	Diameter at 1 inch average	range	Standard deviation	Coefficient of variation
Plot 1:	*Inches*	*Inches*	*Inches*	*Per cent*	*Inches*	*Inches*	*Inches*	*Per cent*
1919	1.05	0 –2.9	0.42	39.6	1.00	(0.25)	0.10	0.10
1924	2.15	0 –5.4	.77	35.8	2.39	1.8–3.1	.36	.15
1929	3.39	0 –7.9	1.20	35.4	3.68	2.1–4.9	.67	.18
Plot 3:								
1919	1.71	.5–3.4	.43	25.1	1.75	(.25)	.10	.06
1924	3.07	1.0–6.4	.82	26.7	3.34	2.6–4.5	.34	.10
1929	4.42	1.5–7.9	1.14	25.8	4.93	3.7–7.0	.50	.10

Just what is the significance of these figures? They evidently show that the average tree of 1919 is gradually getting ahead of the stand average. An old theory which applies perfectly well to unthinned even-aged stands states that the average tree of one measurement gradually falls behind the computed average tree of later remeasurements due to mortality in smaller-size classes, change in the skewness of the distribution curve, and the more rapid development of the larger-size classes. With these thinned plots, mortality in the small-size classes has been eliminated because of the thinning, the skewness of the distribution curve has been affected and, consequently, the trend of the average tree has been disturbed. One might expect that it would maintain its relative position, but it does even more than that; it gains in relative position, as can be determined by constructing a frequency polygon of the values in Table 3 and planimetering the areas above the actual computed average diameter at each measurement and the average of the 35 picked trees. This graph is not included here but it shows that whereas about 42 to 45 per cent of the area lies above the computed average diameter at each measurement for each plot, the 35 trees advance so rapidly that within 5 years after the thinning only about 30 per cent of the area

lies above their average diameter. Such is the evidence that the thinning must affect the average sizes more than the large sizes and allow them to invade and pass the relative positions maintained by diameter groups originally above them. Apparently, therefore, for the type of stand under investigation there is a complete reversal of the theory applicable to unthinned stands.

Another point brought out by Table 5 is that trees originally of the same size within 10 years cover a comparatively large range. One diameter class of 0.25 inch can spread over ranges of 1.3 and 1.9 inches within 5 years and 2.8 and 3.3 inches within 10 years. This variation is somewhat destructive to the ideas regarding the application of average diameter growth rates to diameter classes in certain kinds of stands. The range is also illustrated in the columns headed Standard deviation and Coefficient of variation. The coefficient of variation is defined as the standard deviation over the average diameter. In Plot 1 this coefficient is shrinking, a trend which is also usually noticed in unthinned stands, but in Plot 3 the coefficient is remaining constant. With the 35 selected trees, however, the coefficient increases slightly from an original small value. The range of diameter classes covered by the 35 sample trees when put into percentages of the total range leaps from an original value of 8.6 to 35.4 per cent in Plot 1 and 8.6 to 51.6 per cent in Plot 3. Evidently the development of the stand as a whole does not duplicate the development of single-diameter classes, even if the class be the original average diameter.

These few conclusions drawn from the measurements taken during the early life of a stand cannot be taken as indicative of later events or other age classes. At times they seem to be in direct contradiction to past findings. An unfortunate circumstance is that single trees on the check plot can not be traced, and events transpiring on the thinned plots could not, therefore, be compared to the same events in unthinned stands.

GENERAL OBSERVATIONS

A number of interesting developments on the thinning plots can not be put directly into figures but can be presented only in a descriptive manner.

Of the three plots, plot 3, the one with dominants only, has by far the best appearance. The trees are bigger, look taller since the short trees are all cut out, and have wider and healthier crowns. In the length of crown, however, and in the thickness of the branches the thinned plots appear at a disadvantage to the check plot where the trees are so closely spaced that natural pruning has already begun, and none of the limbs are very heavy. On both the thinned plots the green limbs extend to the ground, and some of them become quite heavy. Hence, to a certain extent, the improvement in the growth of the trees will be offset in the future by the poorer quality of the wood produced. Since the thinned plots have not as yet reached a stage in which the crowns are closing in, there will be still further unfavorable development of the limbs.

CONCLUSIONS

Thinning in small stands has had a favorable effect upon the growth of the trees, and it seems probable that the beneficial effect will last for some time.

A precise spacing in thinning such stands is much less desirable than an approximate spacing in which dominant saplings are left.

Thinning had no determinable effect upon height growth.

Thinning has a decided effect upon diameter growth, over and above the effect of automatically raising the average diameter if the proper trees are left.

Trees in the same initial diameter class do not remain in the same class long but in a very short period cover a wide range of diameters.

Average trees of the first measurement did not remain average trees in later measurements but seemed to gain and be larger than the average. In relative amounts the plot thinned to a precise 8 by 8 foot spacing showed a greater advance, but in actual values the plot thinned for dominants only showed a better advance.

The distribution of the trees in the larger sizes is by far the best on the plot containing dominants only. The distribution curves for all the plots are decidedly warped, but there is evidence of an approach to a more normal distribution.

The thinned stands look much healthier and stockier than the unthinned stand.

Against the advantages of thinning can be placed the disadvantages of increased limb development, which will continue until the crowns close in. By that time the limbs will probably be so heavy that natural pruning will be greatly hindered.

A STUDY OF TEMPERATURE AND HUMIDITY CONDITIONS IN COMMON TYPES OF INSECT REARING CAGES [1]

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INTRODUCTION

Most entomologists who have reared insects have encountered difficulties, particularly with humidity conditions within cages and other containers used to confine the insects. It is well known that tight containers over growing plants become too moist for optimum rearing conditions because of the transpiration of the plants. Condensation on a glass cage is an indication that the dew point has been reached, and that the humidity within the container is very high. It is also well known that material in salve boxes often becomes too dry for optimum feeding conditions for many kinds of insect larvae, and that it is difficult to add just the proper amount of moisture. Under other conditions, salve-box rearings may fail because of too high humidity within, which brings about rapid deterioration of insect food and favors bacterial diseases.

It is customary to have a hygrothermograph in insectaries and to record the temperatures and humidities indicated by the instrument as being the conditions under which the rearings are taking place. The question has arisen in the course of a critical analysis of rearings in connection with the alfalfa-insect project at this station, whether or not such an instrument really indicates the climatic conditions under which the insects in the various types of cages are being reared. If there is a difference, it is desirable not only to know the relative amount and why the difference exists, but also its influence on the insects.

Accordingly, some preliminary observations on this question were made during the early winter of 1926. Further observations were made during the summer of 1927, from July 19 to August 16. The data showed that the temperature within lamp-chimney and screen cages over growing plants was approximately the same as outside the cages or what might be indicated by a thermograph in the insectary, while the humidity was nearly always higher, depending on a number of conditions. A series of atmometer readings bearing on this question was made during the summer of 1929 by Wilbur, as reported by Call (*1, p. 103–105*),[3] which entirely substantiated these conclusions. A more detailed set of parallel observations was made under comparable conditions during the summer of 1930, and this paper is based on these readings, which are given as illustrations.

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[2] The writer was aided by several students in obtaining data on this subject, among whom Everett Ingersoll and Fred Walker should receive special mention. Prof. Donald Wilbur was responsible for setting up the atmometers and for obtaining a set of data during the summers of 1929 and 1930. This paper has been read by several members of the staff of the departments of entomology and physics, and thanks are due for criticisms and suggestions made by them.

[3] Reference is made by number (italic) to Literature Cited, p. 557.

METHOD

The temperature and humidity or the evaporation rate has been taken in the following common types of rearing equipment and compared with the outside:

Lantern-globe cage over growing alfalfa plants or over bare soil, with one or two thicknesses of cheesecloth covers.

Various types of field cages such as cylindrical galvanized iron, covered with fine wire cloth; wooden-frame cages with coarse and fine screen above, or with cheesecloth over the wire, set over plants or bare soil.

A salve-box substitute which consisted of a quart tin fruit can with fairly tight fitting lid, both with and without some alfalfa stems for feeding insects.

A jelly-glass substitute, consisting of an olive bottle set over an atmometer bulb resting on a tin lid with one-fourth inch of moist soil within the bottle, with and without alfalfa twigs. Also, a regular jelly glass with a close-fitting lid was used with thermometers.

Bunch of alfalfa stems in a bottle of water set in a battery jar, covered with a single thickness of cheesecloth.

The temperatures and humidities were obtained from wet and dry bulb thermometers within the cages. For the wet-bulb thermometer a short wick made of several thicknesses of cheesecloth was put through a hole in a cork into a small 10 c. c. bottle of distilled water. The dry-bulb thermometer was suspended beside it. The thermometers used were of a good grade, graduated in either Fahrenheit or centigrade. The check readings were taken from an autohygrometer and a hygrothermograph, though in the tables only the former is considered because the autohygrometer is more comparable to the stationary wet and dry bulb thermometers in the cages.

SOURCES OF ERROR

There are some sources of errors in work of this nature that render data taken by methods described herewith as closely approximate rather than exact.

Differences may be due to the methods of obtaining the relative humidities. Weaver and Clements (*7, p. 262*) state that the stationary psychrometer is of little value. Chapman [4] states that an air movement around the wet bulb at the rate of 5 meters per second and the Food Investigation Board (*3*) states that an air movement of 3 meters per second is necessary for a correct determination of the humidity. The humidities were obtained from the depression of the wet bulb of a stationary thermometer, which is a well-known and standard method (Jameson, (*6*)).

The problem of the best method to be used in a study of this kind proved to be a difficult one. The use of the mercurial thermometer for ascertaining air temperatures is satisfactory. Thermocouple measurements of air temperatures showed larger variations than thermometers because of the delicacy of the instrument and its response to slight variations of air currents. Therefore the mercurial thermometer was used for temperature records.

[4] CHAPMAN, R. N. ANIMAL ECOLOGY WITH ESPECIAL REFERENCE TO INSECTS. 183 p., illus. Minneapolis, 1925. [Mimeographed.]

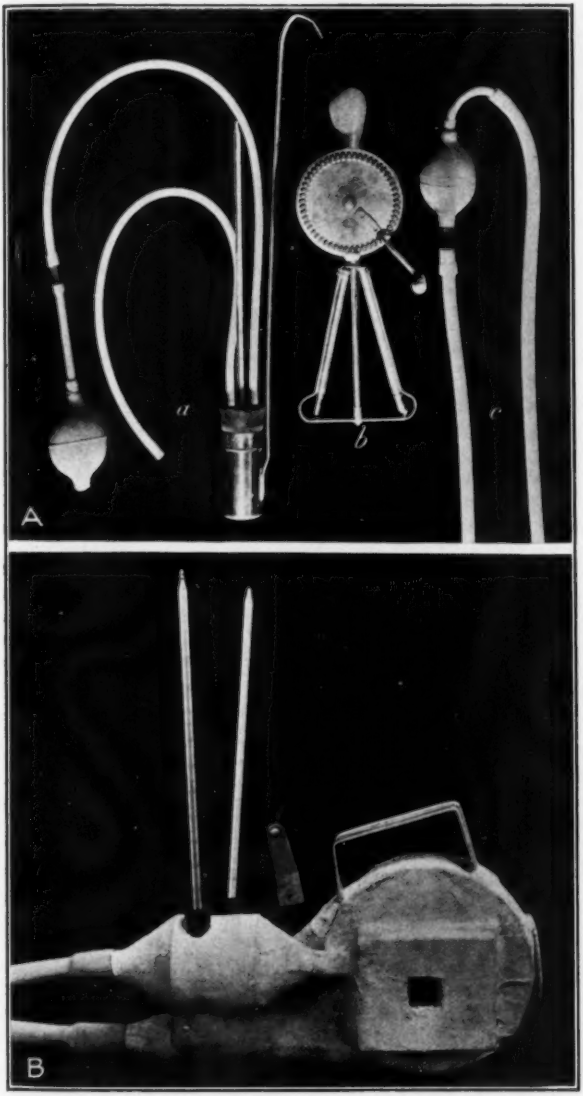

FIGURE 1.—A, *a*, Dew-point apparatus for determining the relative humidity within small, transparent insect-rearing cages; *b*, egg-beater psychrometer; *c*, bulb aerator for small cages. B, Mechanical exhaust psychrometer of original design for determining the relative humidity within the larger insect-rearing cages; the upper tube is the intake, the lower the exhaust, the air being returned to the cage

There appear to be very few suggestions in the literature as to the best method of ascertaining the relative humidity within small inclosed spaces. The sling psychrometer can not be used in cages, except in large ones that can be entered by a person. The egg-beater psychrometer (fig. 1, A, *b*), as designed by Gray (*2*), in comparative tests gave a high degree of conformity to the sling psychrometer in the large cages and in the closed insectary, but in the smaller cages it gave humidity determinations approaching the average of the humidity of the outside and of the interior of the cage. The exhaust psychrometer (fig. 1, B), which is a modified Assmann psychrometer, gave a high degree of correlation with the sling psychrometer when they could be compared, and was found to be satisfactory for the larger cages and where dilution of the cage air with that contained in the instrument did not appreciably affect the readings. For small cages the bulb aerator (fig. 1, A, *c*) was found to be satisfactory. However, in comparative tests of the various methods the Mason form hygrometer, which is a stationary wet and dry bulb psychrometer, gave determinations so closely approximating the ventilated bulb types of psychrometers when left in the cages at least one hour that the writer has used this type by preference. It is necessary to allow the stationary type hygrometers plenty of time to reach final readings; consequently, in these studies, there was a hygrometer of this type in each cage continuously. The small evaporation from the wick could not be detected by comparison with ventilated bulb measurements, and is therefore regarded as inconsequential.

A dew-point apparatus (fig. 1, A, *a*), similar to that illustrated by Marvin (*4*) and Chapman,[5] was made and tested in comparison with the other methods mentioned.[6] This method has the best basis and gives, theoretically, the most exact of all humidity determinations. However, in practice it has yielded larger variations from the sling psychrometer than any other method because of the difficulty of observing the exact time of the formation of the film of moisture and because of the further difficulty of manipulation in closed cages. The hair type of hygrometer, in the comparative tests, was unreliable for both high and low humidities. Calibration was particularly difficult, and the mechanics of this type may introduce added variations from the sling psychrometer.

The writer, therefore, does not concur in statements of the unreliability of the stationary hygrometer for purposes of these studies, except where insufficient time is allowed for the maximum depression of the wet bulb to be attained. For quick determinations an aerated hygrometer is, of course, necessary. Where plenty of time can be allowed for the readings to be reached, the stationary hygrometer, as used in this work, is regarded as the most desirable instrument to use. If an error occurs in the determination of the relative humidity by this method, the results are likely to be too high rather than too low, but in comparative tests this is rarely more than 5 per cent.

It is probable that there are differences in temperature and humidity at various points in the same cage. The recorded readings were taken in close proximity to the growing plants to represent as nearly as possible the conditions under which foliage-feeding forms might be exposed.

[5] CHAPMAN, R. N. Op. cit.

[6] For this suggestion, the writer is indebted to Dr. J. L. Hall, of the chemistry department, Kansas Agricultural Experiment Station. A 12-inch vertical handle soldered on the dew-point apparatus was found to be sstisfactory.

Thermometers are subject to errors, and an error of one or more degrees may result from differences in the thermometers and the reading of them. Before beginning these observations, the thermometers were checked against two high-grade Fahrenheit thermometers, graduated in tenths, and no thermometer used varied more than 1° from the standard high-grade thermometer at the test temperatures 67°, 95°, and 106°. Each variation of a degree Fahrenheit in temperature produces a corresponding variation, but in the opposite direction, of 1.5 to 2 per cent in humidity (*7*).

It was early found that watering potted plants each 24 hours by means of pot bases was preferable to top watering. In all cases the readings were made before watering. The aim in the care of the potted plants was to provide as nearly optimum growing conditions as possible.

A small portion of the wick of the wet-bulb thermometers in the cages was exposed. The evaporation from the wicks and atmometers no doubt raised the humidity within the cages somewhat.

MEASUREMENT OF HUMIDITY

Measuring humidity is one of the most difficult of physical measurements. As far as the writer has found, the best discussion of this problem, as it applies to closed spaces, is a report by the Food Investigation Board (*3*). Unfortunately, this report was not discovered until after the field readings were finished. The greater part of this report is devoted to the methods and devices available for the measurement of humidity. The writers stated (*3, p. 10*)—

> Whilst the unventilated type of wet and dry bulb hygrometer is a notoriously unreliable instrument * * * by modifying the construction so that air at a definite velocity is drawn past the thermometer bulbs, it can be converted into an instrument of satisfactory precision.

They described results with an Assmann aspiration psychrometer, a direct reading type of wet and dry bulb hygrometer, and an aspiration type of wet and dry bulb hygrometer. The latter type (*3, Fig. 11*) could be equipped with a small hand-operated suction fan and adapted to field usage for insect-rearing cages. The writers used a dew-point apparatus as their standard of reference. "The dew-point apparatus stands out conspicuously as being the one instrument which has a sound, theoretical foundation" (*3, p. 18*). The writers concluded that it "works satisfactorily if precautions are taken against draughts, and it affords an efficient means for checking the hair hygrometer." The hair hygrometer is described as a

> simple, direct-reading instrument * * * and whilst its reputation for reliability is not high, yet it serves a useful purpose if employed with care and discrimination. * * * The hair hygrometer, if not subjected to large changes of temperature or humidity, appears to preserve its calibration for several months to within 2 or 3 per cent of the true reading.

DESCRIPTION OF THE EQUIPMENT AND CAGES USED IN THIS STUDY

The autohygrometer which was used to determine the humidity in the open was a Tycos autohygrometer, No. 5558. The hygrothermograph was a standard instrument corrected and standardized at the beginning of each season by use of a sling psychrometer, and by comparison with the autohygrometer at irregular intervals during the season. In the outdoor cage, a Mason's form hygrometer was used.

CAGES

Cages 1 and 2.—Thermometers hung inside and outside the insectary, respectively.

Rearing cage 3.—A lantern globe covered with a single piece of cheesecloth over a clump of growing alfalfa plants. Pot on regular pot base in the insectary and watered at intervals from below or above, according to the experiment. Temperature only.

Rearing cages 4 and 5.—Lantern globe with two thicknesses of cheesecloth, with dry and wet bulb thermometers over growing alfalfa plant in the insectary. Watered as above. Temperature and humidity readings. Wet-bulb thermometer had bulb wrapped with cheesecloth wick reaching into small bottle of distilled water, bottle closed with loose-fitting cork or plug of nonabsorbent cotton.

Rearing cage 6.—Large galvanized screen-wire cage over alfalfa plant in the field, fully exposed. With Tycos hygrometer, Mason's form No. 5532, and Livingston's evaporation bulb.

Rearing cage 7.—Chimney cage in the insectary, as No. 4, with Livingston bulb. Bottle of distilled water buried in the pot so the bulb could be contained in the glass cage with the plant.

Rearing cage 8.—A quart can with Livingston bulb within. Alfalfa branches and insects within to simulate the usual salve box rearing conditions.

Rearing cage 9.—A glass jar with tin lid inverted over Livingston bulb to simulate the jelly-glass type of rearing chamber. Alfalfa branches with some lepidopterous larvae in the jar. About one-half inch of moist soil on the lid of the jar.

Conditions in the open and in the insectary (screened building 6 by 8 by 8 feet) have been considered normal and, therefore, the check on cage data. These data were collected in a season of abnormally high temperature conditions for Kansas. It is believed that differences in temperature and humidity would be more pronounced under such conditions, and therefore any divergence between rearing-cage conditions and outside field conditions would be more clearly indicated.

TABLE 1.—*Temperatures within the various insect-rearing cages, 1930*

Date	Time of day	Temperature by Tycos autohygrometer	Temperature by hygrothermograph	Temperature in cage 3	Temperature in lantern-globe cage 4	Temperature in lantern-globe cage 5	Temperature in outside cage 6	Official Government readings	
								Temperature	Barometer
	p. m.	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	*Inches*
July 7	1.30	98	98	96.8	95	97	----	100	28.80
8	1.30	98	98	93.2	97	98	110	100	28.85
9	1.30	98	99	[a] 89.6	97	97	----	102	28.87
10	1.30	100	100	99.6	98	99	104	102	28.85
11	1.30	101	101	100.4	99	100	103	101	28.77
12	1.30	100.5	100	[b] 101.3	100	100	104	101	28.65
14	1.30	77.5	78	77	78	77.5	85	90	29.07
15	1.30	88	88	86	86	86	96	87	29.03
16	1.30	99	99	95.9	95	94	105	99	28.87
17	1.30	102	103	96.8	95	94	106	103	28.73
18	1.30	105	105	100.4	102	101	109	105	28.65
19	1.30	103	104	98.6	102	101	106	104	28.65
21	1.30	85	85	83.3	84	85	88	88	----
22	4.00	85	85	84.2	85	85	87	85	----
23	1.00	69	69	68.9	69	69	68	66	28.97
24	1.00	92	93	91.5	90.5	92	98	92	28.87
25	1.00	98	98.5	95	94	96	96	99	28.75
26	1.30	102	103	98.6	99	100	104	106	28.72
27	[d] 8.00	87.5	87.5	98.6	101	101	[c] 90	88	28.72
28	1.30	93	93	97	91	92	96	99	28.82
29	1.15	83	83	80.6	82	83	88	88	28.95
30	1.15	79	79	78.8	78	78	80	82	29.09
31	1.15	85.5	85.5	84	83.5	84	91	91	29.05
Aug. 1	2.15	98	97.5	95	94	96	105	101	28.81
2	1.15	104	104	99.5	100	96	106	105	28.64
4	1.15	107	107	104	104	106	111	109	28.75
5	12.00	97	97.5	93.2	94	96	103	98	28.80
6	11.45	98	98	95.9	95.5	96.5	----	99	28.77
Average		94.03	94.23	92.27	92.44	92.85	97.56	96.07	28.82

[a] Plant heavily watered, dying. [b] Plant dead; replaced. [c] 3 to 5 inches of rain. [d] A. M.

The atmometers were exposed at a height near the top of the alfalfa plants in the cages. Wilbur found, as reported by Call (*1*), that there was an average increased rate of evaporation of 1, 29.5, 46.1, 48.9, and 63.9, respectively, from atmometers set at ground level and at heights of 12, 24, 36, and 60 inches above ground.

The figures in Table 1 indicate that the temperature within the lantern-globe cages in the insectary varies about the outside temperature as the mean. It was generally a little lower. The temperature within cage 6 ran a little higher than that of the outdoor temperature fully exposed. The presence of plants in cages 3 and 4 tended to depress the temperature slightly. The larger and healthier the plant, the lower the temperature until individual differences of as much as 7° occurred. As the plants died, the temperature tended to become approximately equal to the outside temperature, which was the condition in cages without plants.

When the cages were in the sun, the readings in the sun in both screen and glass cages ran consistently higher than readings taken at the same time in the shade. The temperatures in cage 6 were almost always higher than readings in the open, as shown by hygrothermograph and official temperatures.

Well-watered soil tended to depress the temperature, whereas dry soil in the pots usually resulted in a temperature similar to outside conditions.

The humidity in the screen cage out in the open (No. 6, Table 2) was nearly always lower than that in well-watered smaller cages within the shaded insectary. The humidity approached that out in the open but ran a little higher generally. The weather conditions appeared to influence the humidity readings in the outside screen cage more than they did those in the cages in the insectary.

TABLE 2.—*The relative humidity within the various insect-rearing cages*

[Barometric pressure, 29.0 inches]

Date	Time of day	Humidity by auto-hygrometer	Humidity by hygro-thermo-graph	Humidity in—		
				Cage 4	Cage 5	Cage 6
	p. m.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
July 7	1.30	43	42.5	89	89	
July 8	1.30	40	40	89	89	24
July 9	1.30	45	42	86	83	36
July 10	1.30	39	38	89	86	39
July 11	1.30	60	56	89	86	39.5
July 12	1.30	39	42	80	89	39
July 14	1.30	36	42	61	48	36
July 15	1.30	37	41	88	92	35
July 16	1.30	32	38	76	85	33.5
July 17	1.30	27	34	70	76	28
July 18	1.30	24	27	74	74	28.5
July 19	1.30	23	29	78	83	28
July 21	1.30	56	56	80	88	58
July 22	4.00	49	50	92	96	60.5
July 23	1.00	75	70	95	[c] 100	95
July 24	1.00	65	67	86	[b] 96	53
July 25	1.00	39	42	79	89	42
July 26	1.30	43	45	83	86	31
July 27	[d] 8.00	56	59	86	80	56
July 28	1.30	35	39	65	85	37
July 29	1.15	52	50	84	85	46
July 30	1.15	53	52	91	96	64
July 31	1.15	49	56	80	96	47.5
August 1	2.15	36	49	82	[c] 69	37.5
August 2	1.15	26	33	70	60	30
August 4	1.15	29	35	84	64	30.5
August 5	12.00	44.5	43	85	73	45.5
August 6	11.45	40	42	82	73	40
Average		42.58	44.98	81.57	82.39	42.22

[a] A light rain falling.
[b] Soil very moist; some condensation on the chimney.
[c] Without plant; soil moist.
[d] A. M.

The humidities in various rearing equipment, as indicated in Table 2, were sometimes more than twice outside humidities. The humidities were increased in the cages by plant growth, by a tighter covering, and by watering. It was common to find drops of water on the sides of the chimneys. The rate of transpiration in plants is reduced at high humidities so that a saturated atmosphere is distinctly unfavorable to plant growth.

A battery jar set over a plant soon became too moist to provide good rearing conditions.

Recognizing the limitations of methods involving the use of thermometers and the sources of error arising therefrom in these studies, it was decided to run a series, using a Livingston porcelain evaporation bulb or atmometer in each of the cages. The results are recorded in Table 3. The rate of evaporation is affected by most all the attributes of climate, so the evaporation rate is a composite index to the main climatic conditions and is dependent upon the following six factors: (1) On the nature of the evaporating fluid; (2) on the temperature of the evaporating liquid; (3) on the humidity of the space into which the evaporation takes place; (4) on the density of the air above the evaporating surface; (5) on the rapidity of the circulation of the air above the evaporating surface; and (6) on the extent of the exposed surface of the liquid (*5, p. 104*). To these should be added that the rate is increased by a decrease in the barometric pressure. Since the atmometers are of uniform diameter and distilled water was used to fill them, the other five factors constituting the variables are the most important attributes of climate.

EQUIPMENT

1. *Insectary check.*—A white surface atmometer on the middle shelf of the screened field insectary. In the sun during mornings of July 7 to 15, and in the shade all day thereafter.

2. *Chimney cage.*—A large flowerpot containing growing clumps of alfalfa. Bottle buried in soil, so that the top of the bottle was at the level of the soil. Glass cylinder over plant and atmometer. Single thickness of cheesecloth.

3. *Salve-box substitute.*—A fruit can inverted over a bulb, a hole in the lid for the stem of the bulb. The lid was held on by friction and was tight. Some alfalfa twigs and insects were kept in cages from July 7 to July 29, after which no plant parts were in the cage. Atmometer always in the shade, on the top shelf about 6 inches from the roof.

4. *Jelly-glass substitute.*—An olive bottle, upside down over an atmometer, resting on a can lid held in place on the bulb stem, about half an inch of moist soil on lid to simulate jelly-glass conditions. Plant portions and insects kept in cage until July 29 only.

5. *Outside check.*—White surface atmometer set near screen cage No. 6, but fully exposed in the sun in an alfalfa field.

6. *Atmometer.*—Set inside of a screen cage, size 36 inches tall, 12 inches in diameter. Framework of galvanized iron. Over a good clump of alfalfa fully exposed in a field; cover kept on.

TABLE 3.—*Evaporation rates in cubic centimeters, in various cages using Livingston's atmometers, July and August, 1930*

Period covered	Actual time	Evaporation in—					
		Insectary check on shelf	No. 2, chimney cage	No. 3, salve box	No. 4, jelly glass	No. 5, outside check	No. 6, screen cage in field
July 7 to 12	121 hours	205	64	(a)	(a)	227	175
12 to 15	70 hours 53 minutes	128	42	9	9	125	91
15 to 19	96 hours	239	77	7	9	259	185
19 to 25	145 hours 35 minutes	158	72	11	8	288	160
25 to 29	93 hours 26 minutes	154	82	12	17	280	200
29 to 31	48 hours 10 minutes	248	66	(a)	(a)	268	205
July 31 to Aug. 6	141 hours 25 minutes	272	158	15	12	420	390
Total	716 hours 29 minutes	1,404	561	54	55	1,867	1,406

(a) Total included in next reading.

Table 3 substantiates the conclusions indicated from Tables 1 and 2. The evaporation was greatest out in the open (cage 5). It was less in the outdoor screen cages. There was no significant difference between the practically saturated closed containers (cages 3 and 4), within which the evaporation was very small.

These atmometers, all of which had a factor of 0.81, varied slightly in the evaporation rate. For example, a test comparison between Nos. 1 and 5 for a period of three days, beginning August 11, the bottles being set side by side in the insectary, showed a difference of 1 c. c. The evaporation from No. 5 was 70 c. c., and that from No. 1, 69 c. c. The differences indicated in this and similar tests were of no real consequence in this experiment so far as the conclusions drawn are concerned.

TABLE 4.—*Summary of atmometer readings in various types of field cages in an alfalfa field, July 21 to August 4, 1930*

Cage No.	Total time	Total evaporation	Type of cage
	Hours	*C. c.*	
5	353.1	375.95	Cheesecloth over ordinary screen, 3 by 3 by 2 feet. Much vegetation.
4	353.45	599.45	Large cage, ordinary fly screen 4 by 4 by 3 feet; door of cheesecloth. Much vegetation.
3	353.45	542.6	Cylindrical screen cage 12 by 29 inches, fine wire 22 to inch. Clump of alfalfa.
2	353.45	420.1	Wooden-frame cage 17 by 17 by 22 inches, ordinary screen covered with cheesecloth. Over grass. Cage set in holes, open around bottom.
1	353.45	515.65	Small cylindrical wire cage 9½ by 12½ inches, 24 wires to inch. No plants.
Check, open field	353	1,055	Bottle and atmometer set in the alfalfa field fully exposed to the sun and wind.

A series of evaporation readings in various types of field cages is recorded in Table 4. These readings indicate that the more open the cage, the more nearly the evaporation rate, and, in consequence, the climatic conditions within the cage approximate the conditions outside (compare cage 4 with cages 1 and 3). The rate of evaporation within cheesecloth-covered cages was markedly less than that in screen cages. Wilbur, as reported by Call (*1*), found that evaporation from an atmometer in a celloglass cage was 85 c. c. During the same time the evaporation from a square cage with fine mesh ranked

next, with 158.2 c. c., that from a large gauze-covered cage was 108.2 c. c., and from an atmometer in the open 224.7 c. c.

CONCLUSIONS

The hygrothermograph set near the cages containing rearing materials indicated very closely the temperatures to which the insects were being subjected, the cage temperatures averaging 1.38° to 1.96° F. lower than the hygrothermograph. It did not indicate the relative humidity, which was 12 to 60 per cent higher in the smaller and tighter cages than in the open, the average difference in cages 4 and 5 being 36.6 and 37.4 per cent, respectively. The temperature within cages in the insectary containing growing plants varied from 8.4° higher to 8° lower than shade temperature as determined by the dry bulb of the autohygrometer. The temperatures in cages in the sun average 3.53° higher than in the insectary, and 2° higher than official temperatures, as recorded by the department of physics, Kansas State Agricultural College.

There was an accumulation of water vapor resulting largely from transpiration of plants in the air of the cages because of the interference to the free circulation of air offered by the screen wire. The wire of smaller mesh allowed less dispersion of the water vapor than the coarser wire; hence, the rate of evaporation from the atmometers in the tighter cages was less than in the more open cage. Temperature differences were negligible factors.

Transpiration of healthy growing plants and evaporation from the surface of the soil tended to depress slightly the temperature in cages and, of course, markedly increased the humidity. The temperatures tended to equalize when the plants became unthrifty and died. The more tightly a rearing cage was closed, the higher the humidity became. Two thicknesses of cheesecloth closed a lamp-chimney cage tightly enough to produce frequently a saturated atmosphere. Since the loss of water from the atmometers in the salve-box and jelly-glass substitutes was so small, the air within must have been almost completely saturated most of the time. Cheesecloth-covered wire-screen cages gave a lower evaporation rate than cages of wire screen alone, indicating thereby a higher humidity within. A bunch of alfalfa blossoms in a cage did not affect the temperature and humidity conditions quite so much as a growing plant. When the sprigs were fresh, the humidity was higher than in the open and the temperature a little lower. As the blossoms wilted, the conditions within the cage approached those of the free air in the shade.

Glass cages, such as a lamp-chimney cage with cheesecloth covers, inverted battery jar, and jelly glass, all showed markedly higher temperatures when set in the sun than when set in the shaded insectary. The humidity at the same time rapidly approached the dew point, and conditions became too moist for most rearings.

These observations emphasized the unsuitability of the salve box and jelly glass for most rearings. The high temperatures and high humidity caused molds, yellowing of foliage put in the cage for insect food, high loss due to bacterial and fungus diseases, and abnormalities of insect behavior and habits in some cases. The presence of moist sand or soil in these cages aggravated the situation, particularly in hot weather.

The temperature and humidity in all the cages were clearly affected by weather conditions. There was a proportionate parallel increase or decrease in both as field conditions varied.

These investigations have indicated the value of the dew-point apparatus for the determination of the humidity in cages. One can look through the glass cages to see the vapor deposit on the silvered chamber, and glass windows may be put in screen or opaque cages for this purpose. A long handle may be soldered or fastened on for use in tall cages. The dew-point apparatus proved to be the most reliable and most usable method of all tried for determining the humidity in cages.

Considerable study was made to determine the best method of ascertaining the relative humidity in small cages. The sling psychrometer, which has been accepted as a standard method, can only be used in cages large enough to swing the thermometers in. The egg-beater type, while equal to the sling psychrometer in the open and very large cages, proved unsatisfactory for the smaller cages, both as to results and operation. An exhaust psychrometer of original design was found to be satisfactory for relatively rapid determinations in the open and in the larger cages. A bulb aerating device proved satisfactory for rapid determinations in small cages. It was found that a dew-point apparatus can be used in small transparent cages, but was subject to large variations because of the difficulties involved in observing the film of moisture on the cup. A hair hygrometer, which indicated the relative humidity directly on a dial, proved to be difficult to calibrate properly and it was inaccurate for high and low humidities. The Mason type hygrometers or stationary wet and dry bulb thermometers which were used in these studies were found to be satisfactory for cage determinations if at least an hour was allowed for the wet bulb to reach the final depression. Readings, when erroneous, are likely to indicate a relative humidity too high rather than too low, though this error is rarely more than 5 per cent.

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THE ASSIMILATION OF NITROGEN BY TOBACCO[1]

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INTRODUCTION

Tobacco as grown in the lower Connecticut Valley is a highly specialized and intensively fertilized crop. From 120 to 200 pounds of nitrogen in commercial fertilizers are applied to the acre. Most of the nitrogen is supplied in cottonseed meal, or other seed meals. There is a rather prevalent feeling among growers that organic nitrogen, especially that of cottonseed meal, is necessary for the growing of the high-quality tobacco leaf demanded by the cigar industry. There is some experimental evidence to support this opinion.

Many field and some greenhouse experiments on the source of fertilizer for tobacco have been conducted in this and foreign countries. There is an extensive literature bearing on the question of forms of nitrogen in their relation to yield and quality of tobacco. No attempt will be made to review the experimental work on this problem, except to point out that there is considerable lack of uniformity in results from different stations. Anderson and Swanback (*2*) [2] report ammonium sulphate as giving a higher yield than nitrate of soda, cottonseed meal, urea, and other forms, but the quality was poor. Ames and Boltz (*1*) report Ohio data showing a higher yield from sodium nitrate than from ammonium sulphate, with little difference in quality. Hutcheson and Copley (*5*) give figures showing that sodium nitrate yielded slightly more than ammonium sulphate, but the quality was slightly better with ammonium sulphate than with sodium nitrate when only single nitrogen carriers were considered. Both yield and quality were lower with organic than with inorganic materials as single sources.

Also, there is now an extensive literature on the question of assimilation by plants of different forms of nitrogen, well summarized by Hutchinson and Miller (*6*), Mevius (*7*), and Prianischnikov (*8*). The reader is referred to these papers for a thorough discussion of the question.

The work reported in this paper was undertaken for the purpose of securing basic facts as to the nutrition of the tobacco plant by nitrogen compounds. It was expected that such knowledge would contribute to the solution of some problems of yield, quality, and cost of production of cigar-leaf tobacco. Some field experiments were made in connection with this research, but most of the work was done in the laboratory and greenhouse, and only the latter is reported here. Most of the studies were made with plants grown in water cultures according to methods generally used by physiologists.

[1] Received for publication Mar. 13, 1931; issued October, 1931. Contribution No. 120 of the Massachusetts Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 567.

METHODS AND PROCEDURE

Since tobacco seedlings are very small and delicate, it was found necessary to develop a special technic for growing the plants in water cultures under sterile conditions. This technic has been described elsewhere (*4*). Plants were also grown in sand cultures, but mostly for the purpose of obtaining supplementary data. The following mineral nutrient medium, a modification of Crone's, was used:

	mol
$Ca_3(PO_4)_2$	0.0022
$CaSO_4.2H_2O$	.0052
$MgSO_4.7H_2O$	.0114
$FePO_4.4H_2O$	.0019
KCl	.0075

To this was added 0.0278 mol $NaNO_3$ or other carrier having an equivalent amount of nitrogen. While the work was in progress Swanback (*9*) at this station proved that a trace of boron was necessary for the normal development of tobacco in nutrient solutions, and 3 parts per million of H_3BO_3 was later added to the stock solution as a standard ingredient. The freshly prepared stock mineral solution had a reaction of pH 7.4, which was only slightly changed by the addition of the several nitrogen compounds.

The nutrient solution was used full strength in the first few series; later it was found that the concentration could be reduced to one-half or one-fourth full strength without materially affecting comparisons, and it was reduced to one-half strength with the series containing large numbers of cultures, to economize chemicals and labor. The concentration of the solution in the sterile cultures was in most cases one-fourth full strength. The unsterilized culture solutions were changed sufficiently often to prevent any detectable change in the nitrogen compounds used. Ordinarily, this was accomplished by changing twice a week. In very hot weather it was necessary to change the solutions more frequently on account of the hydrolysis of urea. The growing period for the plants of the several experiments varied from 53 to 106 days, depending on the season of the year. Most of the experiments ran 70 to 90 days. One of the authors (*3*) has pointed out the importance of extending the growing period in experiments of this kind.

STERILE CULTURES

In the early experiments 3-neck 1-liter Wolff bottles were used as containers. (Fig. 1.) On account of the excessive breakage of these in the steam sterilizer, they were later replaced by 1-liter Erlenmeyer Pyrex flasks, as shown in Figure 2. Mineral solutions and these plus additions of nitrates and ammonium salts were sterilized in the container by steam at 15 to 20 pounds' pressure for 30 minutes. It was found that urea and certain of the other organic materials hydrolyzed with the production of ammonia when sterilized in solution by steam pressure. Therefore, these unstable compounds were sterilized separately by dry heat and introduced into the nutrient solution subsequent to its sterilization by steam. The solutions of the sterile cultures were not changed in the course of the experiments. While it is very likely that more rapid and more normal growth would have

been obtained had these solutions been changed, it was felt that the procedure used was justified on the ground of comparative results.

The list of nitrogen carriers used in both sterile and nonsterile cultures is given below. In the selection of organic compounds, preference was given those amino acids reported as constituents of cottonseed meal. Other materials were used because they represent types of organic compounds, or are found in soils or plants, or have been used by other workers.

Nitrogen Carriers Used

Group 1.—Nitrates: Sodium nitrate, calcium nitrate.
Group 2.—Ammonium salts: Ammonium sulphate, ammonium phosphate, ammonium chloride, ammonium carbonate.

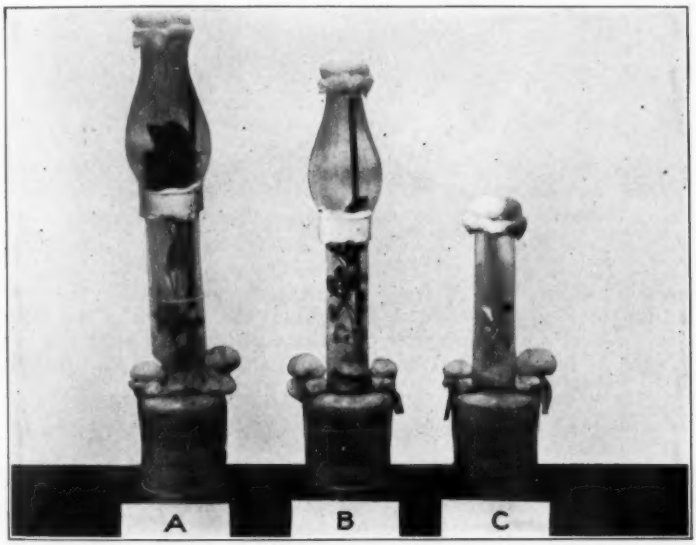

Figure 1.—Sterile sand cultures of tobacco in Wolff bottles with nitrogen carried as follows: A, Sodium nitrate; B, ammonium sulphate; C, minerals only. Similar equipment was used for sterile water cultures for most of the tests

Group 3.—Ammonium nitrate.
Group 4.—Monoamino from monobasic acids: Alanine, cystine, glycine, histidine dichloride, leucine, phenylalanine, tyrosine.
Group 5.—Monoamino from dibasic acids: Aspartic acid, glutamic acid.
Group 6.—Diamino from monobasic acids: Arginine (carbonate), lysine monohydrochloride, lysine dihydrochloride.
Group 7.—Amides: Acetamide, cyanamide, urea.
Group 8.—Aminoamide: Asparagine.
Group 9.—Proline.
Group 10.—Cottonseed meal.

Havana Seed was the variety of tobacco grown. This is not as suitable for greenhouse work as is the Turkish tobacco used by some workers, but it was employed in these experiments because it is the variety commonly grown in Massachusetts.

RESULTS AND DISCUSSION

The data from this study bearing especially on the question of toxicity of ammonium salts have been presented and interpreted in a previous paper (*3*). In that paper evidence was presented to show that sooner or later, depending on the particular salt and its concen-

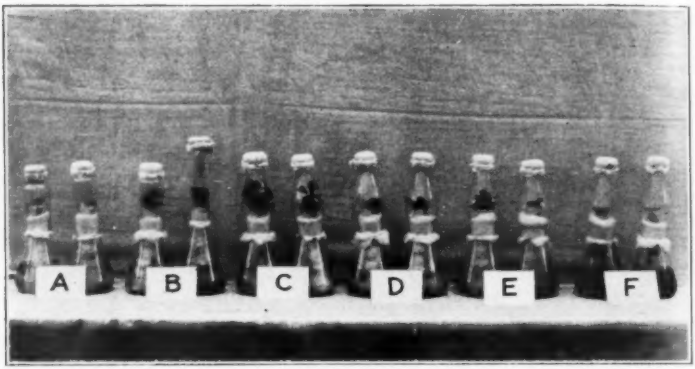

FIGURE 2.—Sterile water cultures of tobacco in Erlenmeyer flasks (adopted for later tests on account of breakage of Wolff bottles in sterilizer). Complete nutrient solution plus nitrogen carriers as follows: A, No nitrogen; B, ammonium sulphate; C, sodium nitrate; D, urea; E, cystine; M, cottonseed meal

tration, toxicity resulted from all the ammonium salts used. Ammonium sulphate was toxic in all concentrations used in the several experiments. These solutions contained nitrogen in amounts varying from as low as 6 to as high as 389 parts per million. Notwith-

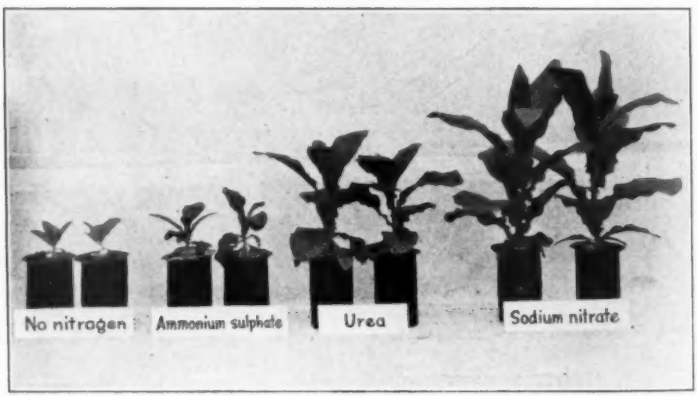

FIGURE 3.—Typical tobacco plants grown in unsterilized water cultures with treatments as shown

standing, the plants grew, and apparently there was assimilation of nitrogen. Toxicity was progressive and cumulative. Table 1 has been adapted from the previous paper, and additional data are presented in Tables 2 and 3. For a discussion of toxicity of the ammonium ion the reader is referred to the paper mentioned (*3*). In Table 4 are given the data obtained from the sterile culture.

Plants grown in urea solutions were intermediate between those grown with nitrates and ammonium salts with respect to size and color. (Fig. 3.) Except in cases where ammonification of urea occurred through hydrolysis or otherwise, the roots were clean and white, and, if anything, better in appearance than those in nitrate solutions. (Fig. 4.)

TABLE 1.—*Dry weight and length of tobacco plants grown in unsterilized water culture with nitrogen from different sources*

EXPERIMENT A

Nitrogen source	Average dry weight of—		Average length of—	
	Tops	Roots	Stems	Roots
	Grams	*Grams*	*Centimeters*	*Centimeters*
Sodium nitrate	12.88	1.87	29.7	30.25
Calcium nitrate	12.86	1.91	27.7	30.5
Ammonium sulphate	2.80	.73	14.5	14.5
Monoammonium phosphate	1.69	.43	10.3	9.8

TABLE 2.—*Dry weight and dimensions of tobacco plants grown in unsterilized water cultures containing various nitrogen carriers*

EXPERIMENT H

Nitrogen carrier	Plants	Average dry weight of—		Average dimensions of—		
		Tops	Roots	Leaves	Stem	Root
	Number	*Grams*	*Grams*	*Centimeters*	*Centimeters*	*Centimeters*
Sodium nitrate	5	6.50	1.06	17.6 by 10.4	17.3	16.7
Calcium nitrate	5	9.15	1.34	18.8 by 10.5	24.8	16.3
Urea	5	4.02	.78	14.1 by 9.1	13.4	13.8
Ammonium sulphate	5	1.09	.22	6.8 by 4.7	6.4	8.8
Monoammonium phosphate	5	1.72	.32	7.1 by 6.3	9.0	9.9
Calcium cyanamide	5	.23	.08	5.1 by 3.0	3.0	7.9
Cottonseed meal	5	.12	.07	4.8 by 2.2	2.7	6.8
Non-nitrogenous minerals only	4	.21	.16	4.9 by 2.4	2.4	22.6

TABLE 3.—*Dry weight of tops, roots, and lower leaves of tobacco plants grown in unsterilized water cultures containing various nitrogen carriers*

EXPERIMENT I

Nitrogen carrier	Plants	Average dry weight of—		
		Tops	Roots	Lower leaves
	Number	*Grams*	*Grams*	*Grams*
Sodium nitrate	14	13.14	2.82	0.14
Ammonium nitrate	9	9.94	2.44	.28
Urea	28	3.39	.96	.135
Ammonium sulphate	95	2.26	.71	.41
Ammonium sulphate+calcium carbonate	10	2.85	.70	.18
Non-nitrogenous minerals only	5	.56	.40	

EXPERIMENT J

Sodium nitrate	18	8.46	1.46	0.08
Urea	17	3.64	.85	.08
Urea *	13	3.18	.68	.12
Ammonium sulphate	42	.945	.215	.075
Non-nitrogenous minerals only	5	.40	.26	.06

* Ammonia found present in latter part of experiment; probably due to excessively hot weather.

TABLE 4.—*Growth of tobacco plants: Sterile water cultures containing various nitrogen carriers*

Nitrogen carrier	Trials	Cultures	Observations
Group 1:	*Number*	*Number*	
Sodium nitrate	2	11	Best growth; 17 to 32 cm.; normal leaves.
Group 2:			
Ammonium sulphate	3	14	Fair growth; 12 to 21 cm.; dark green, roundish leaves.
Ammonium sulphate plus calcium carbonate.	1	5	Fair growth; 7 to 17 cm.; dark green, roundish leaves.
Group 4:			
Alanine	2	6	No growth. Seedlings turned brown and died.
Cystine	3	10	Fair growth; 12 to 16 cm.; normal color.
Glycine	2	6	No growth; yellowish leaves.
Histidine dichloride	1	2	Small growth; 2 to 3 cm.
Leucine	1	3	No growth; 0.5 to 1 cm.
Phenylalanine	1	3	Do.
Tyrosine	2	6	Do.
Group 5:			
Aspartic acid	1	3	No growth; 1 to 2 cm.
Glutamic acid	2	6	Do.
Group 6:			
Arginine (carbonate)	2	6	Slight growth; 2 to 3 cm.
Lysine monohydrochloride [a]	1	6	Slight growth 2 to 4 cm.
Lysine dihydrochloride	1	3	No growth.
Group 7:			
Acetamide	3	9	No growth; 1.5 to 2 cm.; yellowish leaves.
Cyanamide	2	6	No growth; 1 to 2 cm.; yellowish leaves.
Urea	4	16	Good growth; 13 to 32 cm.; slightly dark green leaves.
Group 8:			
Asparagine	2	6	Fair growth; 10 to 20 cm.; mottled leaves.
Group 9:			
Proline	1	4	No growth.
Group 10:			
Cottonseed meal	5	17	No growth; 0.5 cm.; leaves dark green.

[a] At first lysine hydrochloride could not be purchased at supply houses. Consequently lysine picarte and picric acid (as a check) were used. Both were found to be toxic.

TABLE 5.—*Nitrogen fractions in web of tobacco leaves grown in water cultures containing various nitrogen carriers, expressed as percentage of moisture-free weight*

LEAVES NOT DEAD WHEN HARVESTED

Nitrogen carrier	Total N	Water soluble N	Nitric N	Ammoniacal N	Amino N	N present as nicotine	pH of dried plant tissue
Sodium nitrate	4.44	1.46	Trace	0.16	0.76	0.036	5.77
Ammonium sulphate	5.75	2.66	None	.95	1.57	.053	5.08
Ammonium sulphate and calcium carbonate.	5.92	2.31	Trace	.75	1.24		5.43
Ammonium nitrate	5.35	1.95	do	.25	.84	.043	5.56
Urea	4.21	1.56	None	.24	.84	.048	5.17
Check	1.62						

LEAVES DEAD WHEN HARVESTED

Sodium nitrate	1.01						
Ammonium sulphate	3.85	2.62	Trace	1.73	2.56	.046	4.83
Ammonium sulphate and calcium carbonate.	2.00						
Ammonium nitrate	1.64						
Urea	1.25						

Chemical analysis of the leaves throws some light on the question of assimilation of nitrogen. The data of Table 5 show a markedly higher content of total, water-soluble, amino and ammoniacal nitrogen in the sulphate cultures. The ammonium nitrate plants contained these forms of nitrogen in amounts intermediate between those given

calcium and sodium nitrates and those given ammonium sulphate. Urea-grown plants were also intermediate in those respects but nearer the ammonium-sulphate plants than those which received nitrates. The nicotine content, although low as compared with that of field-grown plants, was also highest in plants grown with ammonium salts or urea. The comparatively high content of total nitrogen and fractions, of the dead leaves, is interesting and possibly significant in respect to the question of toxicity. The comparatively low content of nitric nitrogen reported for all treatments can be explained by the fact that only the web (the main part used in making cigar wrappers) of the leaves was analyzed. Later work showed that the midrib and large veins contained larger proportions of nitrate.

As criteria of assimilation of nitrogen in this study the growth characteristics of the plant, the dry matter produced, and the final end-products of nitrogen absorbed by the plant are considered. Water-

FIGURE 4.—Root systems of tobacco plants which received nitrogen from the sources indicated

soluble ammoniacal and nicotine nitrogen are forms which have not been elaborated into proteins and have not been assimilated by the plant. Although the difference between the total and the water-soluble nitrogen was slightly greater in plants grown in ammonium sulphate than in those grown in sodium nitrate, the amount of dry matter produced in the case of sodium nitrate was so much greater as to make the absolute amount of nitrogen assimilated much greater in plants grown with this salt. Also, if to the water-soluble nitrogen all or most of the ammoniacal nitrogen be added as part of the unassimilated nitrogen, both percentage and absolute amounts of assimilated nitrogen are greater in plants grown in sodium nitrate.

Therefore, with these conditions and characters as criteria of assimilation, it appears that the nitrogen of ammonium salts and urea is not as readily assimilated by tobacco as is nitrate nitrogen. Conclusions from all experiments regarding assimilability of nitrogen in the several carriers used are given below.

ASSIMILABILITY OF NITROGEN CARRIERS

Readily assimilated: Sodium nitrate, calcium nitrate, ammonium nitrate.
Assimilated: Urea, ammonium sulphate, ammonium chloride, ammonium phosphate, asparagine, cystine.

Not assimilated: Glycine, alanine, phenylalanine, leucine, tyrosine, histidine dichloride, glutamic acid, aspartic acid, arginine, acetamide, cyanamide, proline, lysine monohydrochloride, lysine dihydrochloride, cottonseed meal.

From these studies it is difficult to account for the failure of ammonium sulphate cultures to produce as good results with tobacco as did nitrates. The results can not satisfactorily be explained by the hypothesis of toxicity of physiologically acid salts advanced by Prianischnikov (*8*), nor by the results of Mevius (*7*) with maize. For further discussion of the question of the relation of reaction of medium to toxicity of ammonium compounds the reader is referred to another paper (*3*) by one of the present authors. However, these results can be explained in part at least on the basis of assimilation of nitrogen. Poor assimilation means improper metabolism, the cells become weakened and open to the attack of any parasitic or saprophytic organisms present in the media. In the case of roots, they decompose or rot; the leaves dry up and fall off.

As for assimilation of the nitrogen of cottonseed meal, it appears that there is very little in the meal that tobacco can assimilate directly. In unsterilized cultures the plants made practically no growth and developed symptoms very similar to those observed when ammonium sulphate was used. In the sterile cultures there was no growth. Only one of the amino acids of cottonseed meal produced any significant growth, and that, cystine, is present in cottonseed in only small amounts. Some of the amino acids and amides studied produced symptoms somewhat like those caused by ammonium salts, but not enough plant material was available for chemical studies of assimilation.

It seems, then, from this study that cottonseed meal in the unchanged form or with its chemical constituents hydrolyzed, contains little nitrogen that the tobacco plant can utilize directly. Further, it appears that best results may be expected in the field only when conditions are right for nitrification.

SUMMARY AND CONCLUSION

In studies of the assimilation of forms of nitrogen by Havana tobacco, 2 nitrate salts, 4 ammonium salts, ammonium nitrate, and 17 organic nitrogen compounds were used. Cottonseed meal being the principal carrier of nitrogen in Connecticut Valley tobacco fertilizers, preference was given those amino acids reported as constituents of cottonseed meal in the selection of organic carriers of nitrogen. Cottonseed meal itself was used. Several series of plants were grown in nonsterile and sterile water and sand cultures. Quantitative data on growth and yield were obtained from the unsterilized water and sand cultures only; qualitative data from the sterile cultures. Analytical data on the chemical composition of the web of the leaf were obtained for plants grown in nonsterile water cultures.

Taking yield data, growth characteristics, and chemical composition as criteria of assimilation, it is concluded that of the various nitrogen compounds studied, the nitrates are most readily assimilated by Havana tobacco. Urea, ammonium salts, asparagine, and cystine are next, in order, to be assimilated. From data accumulated it appears that the toxicity of ammoniacal nitrogen can be explained only partially by the hypothesis of physiological acidity advanced by Prianischnikov or on the grounds of hydrogen-ion concentration

used by Mevius. The authors advance the hypothesis of improper metabolism resulting from imperfect assimilation of nitrogen as an indirect partial cause of the toxicity.

Of the various amino acids reported as present in cottonseed meal, only one, cystine, showed any appreciable assimilability. Cystine is said to be present in cottonseed in only small amounts. Cottonseed meal itself, or its hydrolyzed products, gave no evidence of being assimilated in the unchanged form. Evidence points to the need of ammonification and nitrification of this organic nitrogen carrier before it can be fully assimilated by Havana tobacco.

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